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The Editor's Viewpoint 341

Relative Merits of Vacuum Investing of Small Castings as Compared to Conventional Methods. *R. W. Phillips* (Indiana Univ.) 343

The Effect of Wetting Agents on Oral Lactobacilli. Preliminary Report. *F. E. Walter* and *H. J. Carlson* (Western Reserve Univ. and Babies and Childrens Hospital, Cleveland) 353

Tryptophane in Carbohydrate Metabolism. *N. C. Turner* and *G. E. Crowell* (Boston, Mass.) 359

Penicillin and Caries Activity. *H. A. Zander* and *B. G. Bibby* (Tufts College Dental School) 365

Effects of Topical Sodium Fluoride in the Mandibular Teeth. *A. L. Russell* (State Board of Health, Pierre, South Dakota) 369

A Determination of the Number of Surfaces Involved in Carious Extracted Teeth. *K. E. Wessels* and *V. D. Cheyne* (State Univ. of Iowa) 375

The Role of Oxalates in Rat Dental Caries. *C. A. Schlack, S. R. Howell, B. L. Taylor, V. J. Berzinskas* and *M. H. Aborn* (National Naval Medical Center, Bethesda, Maryland) 383

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, W. J. Gies, R. G. Hutchinson, Jr.; T. P. Hyatt, Milo Hellman, A. H. Merritt, B. B. Palmer, P. R. Stillman, L. M. Waugh.

General Meetings have been held on this schedule: (1) New York, Dec. 21, '22. (2) Chicago, Mar. 7, '24. (3) Chicago, Mar. 20, '25. (4) Chicago, Mar. 26, '26. (5) New York, Apr. 19, '27. (6) Washington, Mar. 25–28, '28. (7) Chicago, Mar. 23–24, '29. (8) Toronto, Mar. 24–26, '30. (9) Memphis, Mar. 21–22, '31. (10) Columbus, Mar. 19–20, '32. (11) Chicago, Mar. 18–19, '33; adjourned executive session, New York, May 25, '33. (12) Chicago, Mar. 17–18, '34. (13) Chicago, Mar. 16–17, '35. (14) Louisville, Mar. 14–15, '36. (15) Baltimore, Mar. 13–14, '37. (16) Minneapolis, Mar. 12–13, '38. (17) Cleveland, Mar. 18–19, '39. (18) Philadelphia, Mar. 13–14, '40. (19) St. Louis, Mar. 15–16, '41. (20) New York, Mar. 14–15, '42. (21) Chicago, Mar. 13–15, '43. (22) Chicago, Mar. 18–19, '44. (23) Chicago, May 27, '45. (24) Kansas City, Mar. 16–17, '46. (25) Chicago, June 21–22, '47.

Objects (*Const., Art. II*): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

Membership: total (Nov. 10, 1946), 627. *Eligibility* (*Const., Art. 14, Sec. 1*).—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to stomatology, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund of the International Association for Dental Research (as of Mar. 16, 1946): $1,402.98. There have been no expenditures from this fund.

Divisions may be organized by the members in any nation. There are now divisions in Canada, China, England, Germany, Hungary, Palestine, South Africa, and the United States—8. *Sections* may be organized by the members in geographic centers within the divisions. There are now sections (32) in Vienna ('29); Halifax ('28), Toronto ('21), Winnipeg ('30); Chengtu ('34); Prague ('32); London ('31); Budapest ('34); Johannesburg ('34); Palestine ('39); Ann Arbor ('23), Baltimore ('33), Boston ('20), Chicago ('20), Cleveland ('30), Columbus ('32), Detroit ('37), Houston ('39), Indianapolis ('41), Iowa City ('36), Louisville ('32), Minnesota ('28), New Orleans ('37), New York ('20), Philadelphia ('28), Pittsburgh ('28), Richmond ('33), Rochester ('33), St. Louis ('28), San Francisco ('24), Tennessee ('44), Washington, D. C. ('31). The names of the secretaries are given on p. 2 of this cover.

Journal of Dental Research: The Association's official publication. See register of officers and editors on page 2 of cover.

THE EDITOR'S VIEWPOINT

Phillips (343) has carefully evaluated vacuum investing and conventional methods for small castings. Such work gives a sense of justification to the men who use investing methods. We occasionally receive letters of inquiry about the possibility of publishing papers on physical research in this *Journal*. The policy is to publish the results of original and honest investigation in the fields of knowledge pertaining to the teeth and mouth. The Editor makes no distinction between types of investigations but attempts to find space for all well conducted, honest investigations.

Walter and Carlson (p. 353) appear to have entered on an interesting phase of possible caries control. While they use trade names to cite their wetting agents it appears permissible in this instance because many are of secret formulae and others are mixtures. The stimulating effect of some may be due to use of sugar as a flavoring agent or may be the direct result of the wetting agent. The authors promise further results.

Turner and Crowell (p. 359) continue their study of starch reduction and dental caries by feeding tryptophane to a small group of young volunteers. Obviously, as the authors admit, the group is too small for statistical evaluation and sweeping conclusions. Their results, however, encourage further study of this factor in caries control.

Zander and Bibby (p. 365) found that penicillin appeared to interfere with acid production in saliva and that it markedly reduced or prevented caries in hamsters. Their suggestion that this method is impractical for man is open to question. If penicillin is as effective in man as it was in their hamsters, it may become useful in man as the economic barrier is reduced and as new antibiotics are developed which reduce the importance of the effects of penicillin on the oral flora.

Russell (p. 369) has applied certain statistical yardsticks to the data of Knutson and Armstrong in an effort to explain the apparent relative ineffectiveness of topically applied fluorides on mandibular teeth. He contends that the reduction in caries was greater than that reported by Knutson and Armstrong because of failure to fully evaluate caries reduction by fluorine leakage to the control quadrant. It has long appeared to us that Arnim's compilation of Leigh and Hyatt's data (*J. D. Res.* 21:243, 1943) explains many of the seemingly peculiar results in caries control. Many individuals have attempted to explain the relative protection of anterior teeth on the basis of flow and contact of fluorine but Arnim's illustrations demonstrate that teeth have varying caries attack rates, beginning with the readily attacked first and second molars and ending with the mandibular anterior teeth. If the caries is controlled, or reduced, the greater amount of protection will appear to be in those teeth less often attacked by caries, i.e., the anterior teeth and the mandibular teeth.

Wessels and Cheyne (p. 375) studied 104 permanent teeth and 273 deciduous teeth to determine the number of surfaces involved by caries in extracted teeth. Their results indicate that the method of assigning 5 points (surfaces) to missing teeth is somewhat of an exaggeration of the true picture in patients of the University of Iowa Clinics. Perhaps, some dentists in private practice have accumulated enough teeth with adequate data to present the proper values for other types of individuals. Obviously, the difference would be great between dentists who extract all teeth with advanced caries and those who are more conservative.

Schlack, Howell, Taylor, Berzinskas and Aborn (p. 383) were unable to reduce rat caries by the feeding of oxalates although this method had reduced erosion (acid decalcification) in rats. They state,

"This work tends to minimize the importance of the acidogenic factor in the initial stages of the carious process." It is questionable that one can follow the induction: Oxalate deposit inhibits acid decalcification in rats fed acid containing beverages. Oxalate deposit does not inhibit caries in rats. Therefore, caries is not initiated as a decalcification. We might ask, Is it not possible that oxalates inhibit the mass action by acid brought into the oral fluids by drinking water and fail to inhibit those concentrations of acids produced in specific sites by microorganisms?

H.B.G.R.

RELATIVE MERITS OF VACUUM INVESTING OF SMALL CASTINGS AS COMPARED TO CONVENTIONAL METHODS[1]

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The object of any dental casting technic is to produce a casting which will fit the preparation properly and also be smooth and entirely free from bubbles and surface irregularities. Not only must rough castings frequently be remade but also attempts to seat castings containing even very minute bubbles can result in damage to the preparation. Thus any casting procedure must be evaluated by its ability to produce castings which are free from surface imperfections and which are accurate in their fit.

It is a well established fact that surface roughness, such as nodules, is largely due to faulty investing of the wax pattern. Bubbles on castings are caused in 2 ways: 1. air incorporated in the investment itself and 2. air trapped on the surface of the wax pattern during the investing procedure (1). Much time and effort have been spent in the past in an attempt to devise investing technics which would eliminate, or at least minimize, the presence of these air bubbles. The development of mechanical spatulation and the use of certain wetting agents have aided tremendously in the elimination of surface irregularities.

It is definitely true that a standardized investing procedure employing the proper water-investment ratio, mechanical spatulation, a thin coating of a wetting agent, and careful painting of the investment on the pattern can consistently produce perfectly smooth castings. However, the necessity for absolute adherence to a very careful and standardized technic can not be over-emphasized. The operator must have a working knowledge and an appreciation of the importance of all the factors involved.

Recently the use of vacuum equipment for investing has received increasing prominence. Particularily during the war years did vacuum processes make their greatest growth. Many industrial companies found that small and intricate metal parts needed in war production could best be made by the use of wax patterns and the conventional dental casting method. Speed in production and a close tolerance made surface smoothness essential. Therefore, vacuum apparatus was used as an aid for securing smooth multiple castings. As an outgrowth of this industrial use of vacuum equipment, dentistry has adopted the principles and although its use in dentistry is not yet wide-spread, it is growing in popularity. Granger (2), Estes (3), Ray (4), and others have published re-

[1] Read at the 25th general meeting of the International Association for Dental Research, Chicago, June 21 and 22, 1947. Received for publication June 28, 1947.

ports on its use in dentistry. At the present time there are at least 4 commercial types of vacuum equipment designed especially for dental use as well as various other inexpensive apparatus which make use of compressed air or water aspirator.

The underlying principles involved in the vacuum investing process are relatively simple. The air in the mix of investment is eliminated by being subjected to a high degree of vacuum. Most of the air bubbles merely expand until they are able to move up through the mix. The remainder of the dissolved air is washed out by the rapid evolution of water vapor, since water actually boils at the high degree of vacuum used. (Water boils at 60°F under a vacuum of 29.5 inches.) After the air has been removed, the vacuum is held for a few moments, then broken and air is readmitted into the bell jar. The atmospheric pressure forces the investment into very intimate contact with the surface of the wax pattern, condensing any water vapor bubbles which might be present.

PURPOSE

There are many conflicting statements being made, both pro and con, concerning vacuum investing. To date, no one has comprehensively studied all of the factors involved or attempted to compare conscientiously the relative merits of vacuum investing with that of a careful hand technic. It was for that purpose that this investigation was undertaken.

PROCEDURE

The particular vacuum apparatus used in this experiment was one employing a motor driven pump with a 10 inch plastic bell jar.[2] All results are based upon the use of this machine. Other types of vacuum equipment behave similarly, but they should be investigated before a general statement is made. This study deals entirely with small castings; observations with large castings are to be reported in a future paper.

In the use of this vacuum apparatus, the manufacturer's directions were closely followed. The single evacuation technic was employed. A thin coat of wetting agent[3] was painted on the pattern immediately before the investment was placed in the ring. Experimentation proved that this, or a similar wetting agent, is a valuable aid in obtaining routinely smooth castings by vacuum. The investment was mechanically spatulated for 70 turns and was then poured around the mounted pattern. A slight excess (approximately ½ inch) was added in the rubber extension tube. The ring was placed under the bell jar and vacuum applied. Ten seconds were allowed for reaching vacuum, the vacuum then being held for 10 seconds. Air was then reintroduced into the bell jar rather rapidly in order to facilitate removal of all bubbles. During this entire process, the ring was vibrated by striking the platform, which is mounted on springs. Vibration is essential to prevent the investment from foaming out of the extension tube and to aid in its return into the ring.

Castings made from patterns invested in this manner were compared to those

[2] Kerr Vacuumatic made by the Kerr Manufacturing Company, Detroit, Michigan.
[3] Vacufilm recommended by Kerr Manufacturing Company, Detroit, Michigan.

invested by a careful standardized hand technic. As much as possible, all the variables were standardized for both technics, such as the water-investment ratio, method of mixing the investment, burn-out time, etc. In the hand technic, the investment was carefully painted on the pattern. The ring was filled and the mounted pattern, fully covered with investment, was then gently vibrated by hand down into the investment. This hand technic was so perfected by 2 different operators that results could be duplicated on every casting.

For comparing the 2 outlined technics, some 800 experimental castings were made in the course of this study. Any significant differences observed by one investigator were always checked by a second, working independently. Only those observations which were found by both workers are reported in this paper.

TIME REQUIRED TO INVEST

The time required to invest a single pattern by the 2 methods was compared. The stop-watch was started when the investment was first added to the water and was stopped at the completion of the investing procedure. No difference in investing time between the 2 methods could be found, 2 minutes, plus or minus a few seconds, being required for both procedures. Thus, for single castings, neither one is advantageous insomuch as saving time is concerned.

EFFECT ON WATER RATIO

One of the first factors to be considered in the use of vacuum apparatus is the effect on the water-investment ratio. The actual boiling of the water under high vacuum, accompanied by the rapid evolution of air from the mass might lead one to believe that the water loss is great enough to upset seriously the recommended ratio. The water loss was measured by 3 different methods. 1. From a vessel containing water (15 cc.) alone. 2. From a plaster bowl containing the mixed investment and water. 3. From the ring and extension tube containing the mixed investment and water. In all cases the vessel and contents were accurately weighed, immediately transferred to the vacuum apparatus and subjected to the routine procedure described previously. Then the vessel and contents were again rapidly weighed. In all 3 methods the water losses compared very closely.

The average water loss on a total of 24 individual tests was .32 gram, or approximately $\frac{1}{3}$ cc. This loss is not sufficient to alter seriously the physical properties or working characteristics of any investment. As a further check, the setting time was studied. A Gillmore needle was used to make this measurement. Readings were taken both at the top and at the bottom of the ring since it might be reasoned that the water content would be higher in the upper portion of the investment. No difference was found in the setting time between these 2 portions and no difference between vacuum and hand invested specimens. This again indicates that the water content is not appreciably altered.

TEMPERATURE CHANGE IN WAX PATTERN

The second factor to be considered is any temperature change in the pattern during evacuation. The heat of vaporization of water is 585 calories per gram

at 75°F, which means that 585 calories of heat will be absorbed for each gram of water which is vaporized. Naturally, a marked cooling effect and drop in temperature of the wax pattern could result in contraction of the wax and an improperly fitting casting. Actually, however, if only .3 gram of water is lost during evacuation by this particular technic, then only 175.5 calories would be absorbed and the cooling effect would be quite small. In order to verify this assumption, a chromel-constantan thermocouple was imbedded in the wax pattern and the temperature change during evacuation was measured by a Leeds-Northrup Model K potentiometer.

The average drop in temperature was −2°F, which is not of sufficient magnitude to be practically significant. This figure compared closely to that of Ray (5). This is, of course, under what might be considered a normal operating condition. If the evacuation is unduly prolonged, the temperature drop can go as high as −7°F.

EFFECT ON DENSITY

Since the use of vacuum apparently does remove most of the air from the mass of investment, it would seem logical to assume that the density of the investment would be greater than when manipulated by hand. This factor was studied by means of a wet test gas flow meter, shown in *fig. 1*. The density is determined indirectly by measurement of the flow of air in cubic feet per minute through a plug of investment. The denser the investment, the less will be the air passing through per unit of time. The air going into the investment plug was held under a constant pressure of 15 pounds.

The results, on 3 different investments, are shown in Table I. It can be seen that for each investment the amount of air passing through the investment plug is always greater for the hand manipulated specimen. In fact, for investment B, the amount of air flow in the hand manipulated specimen is over twice that of the vacuumated one. Undoubtedly then, a denser mass of investment does result from the use of vacuum, due of course to removal of air voids which increase the porosity of the investment.

This greater investment density results in some increase in strength. The comparison of the 7 day crushing strength, in pounds per square inch, for the same 3 investments is shown in Table II. These figures represent an average of at least 12 individual specimens. Each of the 3 investments shows an increase in strength when manipulated by vacuum, although none is tremendously greater; for investment C the difference might well be within experimental error.

SMOOTHNESS OF CASTINGS

The most controversial issue in this whole problem, of course, is whether smoother castings can more consistently be obtained with the use of vacuum equipment. From a microscopical study of 800 castings made in the course of this investigation, it is known that vacuum investing, properly done, will produce castings that are routinely free from bubbles. Perfect smoothness is the rule and a nodule is the exception. However, in all fairness, the statement made at

the first of this paper must be reiterated, i.e., a careful hand technic can be developed which will *also* consistently produce perfectly smooth castings. If an individual has not perfected or mastered a technic, if he has not been properly trained in the importance of a standardized technic, then vacuum investing definitely can be a valuable aid. The human element is reduced and smooth castings can be obtained consistently regardless of the experience or perfection, within limits, of the individual. It would be false though to state unequivocally

Fig. 1. Wet test flow meter used to measure density of investment, used through the courtesy of J. M. Ney Company.

that vacuum investing is necessary for routinely producing smooth castings, since a careful hand technic can do the same.

SURFACE CONDITION OF CASTING

Since castings without such imperfections as bubbles can be produced consistently either by hand or by vacuum, the only other related factor to be considered is the actual character of the gold surface itself, irrespective of surface nodules or bubbles. A definite difference was noted in the microscopical study of these castings made by the 2 methods.

Fig. 2 shows a gold plug made from a wax pattern in which the top half was

invested by hand and the bottom half by vacuum. There is a very obvious visual difference between the surface condition of the 2 halves. The side invested by vacuum appears smoother and denser, even though both are entirely free from bubbles. This difference is more evident in the photomicrographs of these areas. *Fig. 3* shows the microscopical picture (150×), unetched, of the hand invested side, while *fig. 4* shows the vacuum invested half, under the same magnification. Obviously, the vacuum did produce a denser surface. Of 5 investments tested, 4 showed this visual difference but on the fifth investment the areas were identical. Of course, this is entirely a surface phenomenon and does not mean that the density of the entire casting is any greater when invested by vacuum. This denser gold surface is due to the reduced porosity of the adjacent investment, as was shown in the air flow tests. Whether this finding

TABLE I

INVESTMENT	AIR FLOW (CUBIC FEET PER MINUTE)	
	Hand	Vacuum
A	.014	.009
B	.018	.008
C	.012	.009

TABLE II

INVESTMENT	CRUSHING STRENGTH (7 DAYS)	
	Hand	Vacuum
A	1,530	1,870
B	2,300	2,510
C	1,110	1,220

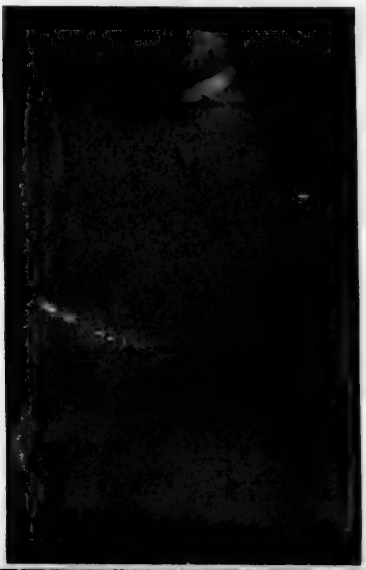
has any practical significance is difficult to say. It probably would be unimportant on the external surface where it would be obliterated by the polishing operation, and its importance on the cavity side is debatable. It is sufficient to say at this time merely that the difference is evident and its practical importance is unknown.

DISTORTION OF PATTERN

Another factor to be considered is whether there is any distortion of the wax pattern during the vacuum process. Many individuals believe that the vigorous elimination of air from the investment and the sudden release of vacuum with the accompanying return of atmospheric pressure might seriously distort the wax pattern. This factor was checked by over 400 castings, using MOD and full crown steel dies, 2 and 3 surface preparations, and three quarter crowns on technic teeth.

In any investigation which evaluates a casting procedure, control of pattern

FIG. 2.



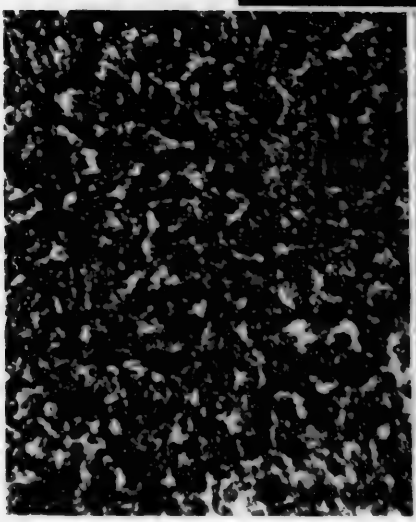


FIG. 3.

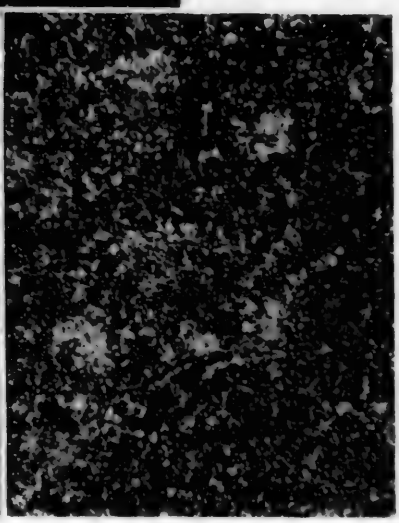


FIG. 4.

Fig. 2. Gold plug cast from wax pattern in which top half was invested by hand and bottom half by vacuum.

Fig. 3. Photomicrograph (150×) of the surface of hand invested half of plug.

Fig. 4. Photomicrograph (150×) of the surface of the vacuum invested half.

distortion is most important. The ability to reproduce accurately in gold an intricate wax pattern is to a large degree dependent upon minimizing distortion of the wax pattern during its removal from the mouth and throughout the in-

vesting procedure. Many factors play a part, such as manipulation of the wax, spruing, length of time and temperature at which the pattern may be stored, etc. It is not the purpose of this paper to discuss this phase of the casting process. However, the hand technic used in this study was so standardized in this

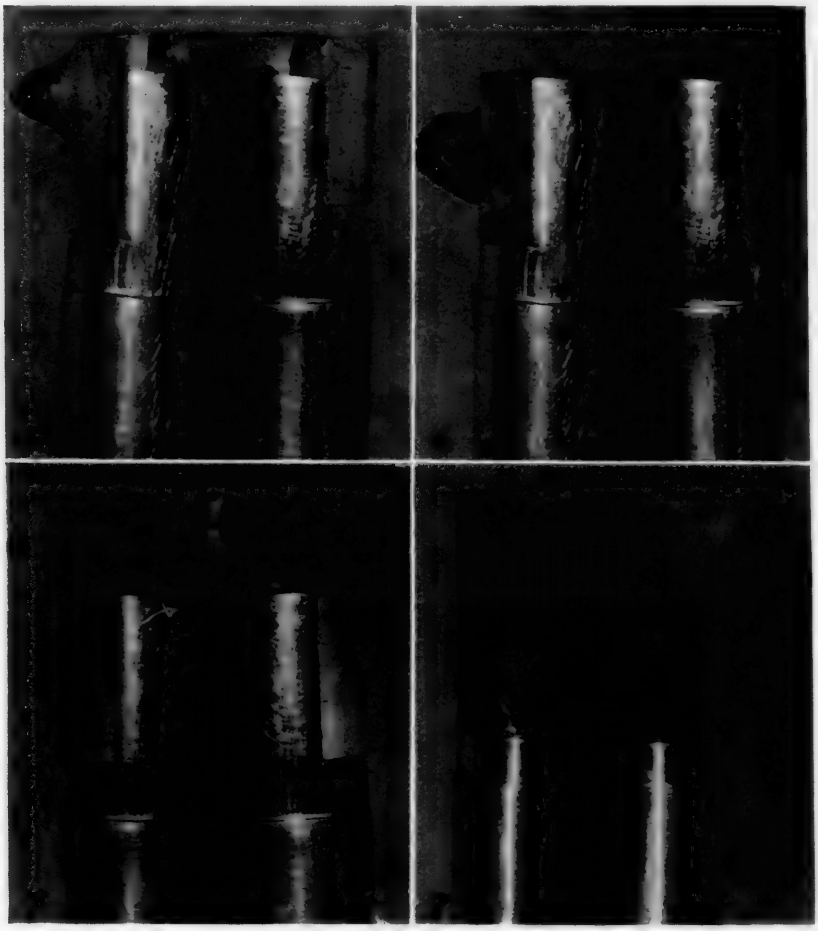


Fig. 5. Distortion induced by vacuum on steel MOD die.
Fig. 6. Casting invested by hand showing no distortion.
Fig. 7. Casting invested by vacuum except sprue was attached on the occlusal surface.
Fig. 8. Full cast crown shows no distortion.

respect that regardless of how many castings were made for a particular die, each and every casting fit accurately. Thus, when this same technic, except for the vacuum method of investing, produced even a slight inaccuracy in fit, it must be attributed to the influence of the vacuum. (In this experiment, a

casting was considered to be distorted when by visual observation it did not fit accurately on all margins.)

The only evidence of distortion occurred on the steel 3 surface die. *Fig. 5* shows an example of what occasionally does happen with the use of vacuum on this particular preparation. The degree of distortion varied, sometimes being only slight and sometimes severe as shown here. The casting should fit as shown in *fig. 6*, where the technic was identical except for hand investing.

It should be emphasized that the frequency of the distortion on this die with vacuum is rather spasmodic. It may occur 3 times in succession and then not at all for 5 or 6 castings. The average for this investigation would be approximately 1 out of every 3. Undoubtedly the cause of the distortion is a bending of the proximal wall as the weight of the investment and atmospheric pressure are

Fig. 9. Such typical dental preparations show no distortion.

forced against it. It occurs in approximately 1 out of every 3, regardless of whether the vacuum is broken rapidly or very slowly. Whether distortion will or will not occur is apparently governed by the manner in which the air and investment do strike this long proximal wall as they reenter the ring. Distortion will never occur if the sprue is attached to the pattern on the occlusal surface instead of the proximal wall, as shown in *fig. 7*. With this sprue arrangement, the casting is more or less a straight plug, having no unsupported walls lying at right angles to the weight of investment and air. On full cast crowns, as in *fig. 8*, the walls are all connected and support each other. Consequently, there is no wax pattern distortion.

On the 2 and 3 surface preparations made on technic teeth, *fig. 9*, which are typical dental preparations, I could find no distortion, even when sprued on

the proximal surface. Of course, the walls on these preparations are not so long as those of the steel die and are thus less subject to distortion.

Again it is difficult to determine the practical significance of this finding. On most typical dental castings, no distortion will occur with the use of vacuum regardless of the place at which the sprue is attached. However, if the preparation has exceedingly long walls, approaching those of the steel MOD die, a safety precaution in the use of vacuum would be to sprue on the occlusal surface rather than on a proximal side.

There are many other factors which must be studied, such as the use of vacuum in investing large castings, pouring of models, oil impregnation of stone dies and models, different types of vacuum equipment, etc.

CONCLUSIONS

1. On the basis of some 800 experimental castings, it can be said that smooth small castings, free from bubbles and nodules, can routinely be produced either by use of vacuum equipment or by a careful hand technic. However, for an inexperienced or inept operator such results are easier to obtain with vacuum apparatus, since the human element is reduced. The time necessary to carry out the investment procedure is the same for both methods.

2. Vacuum investing produces a denser mass of investment, as measured by an air flow meter, which results in slightly greater crushing strength of investment.

3. The increased density of the investment in turn produces a denser gold surface. This is entirely a surface condition and the practical significance is not known.

4. The water-investment ratio and setting time are not altered appreciably by the use of vacuum. The temperature change in the wax pattern is only $-2°F$ under normal operating conditions.

5. The only evidence of distortion caused by vacuum occurred on the steel MOD die, in approximately 1 out of every 3 made. This can be eliminated on this particular die by spruing on the occlusal surface rather than on the proximal wall.

The author wishes to acknowledge the very capable assistance of Mr. James O. Stewart, who conducted many of the technical tests in this investigation.

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THE EFFECT OF WETTING AGENTS ON ORAL LACTOBACILLI[1, 2]

Preliminary Report

FRANK E. WALTER, B.S. and HARVE J. CARLSON, Dr.P.H.

From the Department of Pediatrics, School of Medicine and from the School of Dentistry, Western Reserve University and Babies & Childrens Hospital, Cleveland, Ohio

The introduction within the past few years of a large number of surface-active agents has stimulated numerous explorations into the properties of these compounds. In the field of bacteriology investigations of their inhibitory and stimulatory effects have been made on a variety of organisms. The work of Dubos (1) on *Mycobacterium tuberculosis* and work in this institution on *Staphylococcus aureus* and *Escherichia coli* and other organisms (2) have prompted the present study. It was originally intended to seek a method to produce more rapid, homogenous growth of *Lactobacilli* in liquid media. The work has since evolved into several phases.

The object of this paper is to report 1. the effects of minute amounts of various types of wetting agents on stimulation of growth of *Lactobacilli* in both liquid and solid media, and 2. the inhibitory activity of some of these agents.

Methods. Strains of recently isolated oral *Lactobacilli* were used throughout the study. Cultures were transferred in broth every 24–48 hours and to solid media once each week. In testing surface-active agents for inhibition and stimulation the Oxford cup method was used. Two ml. of a 48 hour culture of oral *Lactobacilli*, previously diluted 1:1000, was used to seed 1 liter of tomato juice agar.[3] After the shake cultures were poured into sterile petri dishes, the agar surfaces were allowed to dry for 30 minutes.

Sterile porcelain cylinders were placed on the surface of the agar and filled with sterile dilutions of the surface-active agents and controls of the diluting fluids. The plates were incubated at 37° C. for 24, 48, and 72 hours. Zones of partial or complete inhibition and stimulation of growth of the organism were measured in millimeters. Dilution series were prepared of the surface-active agents to ascertain their stimulatory characteristics in liquid media. Lactose, nutrient, nutrient with 2% dextrose, and bacto-dextrose special[4] (pH 5.2) broths were used. With the exception of the last-named medium, these were made up with sterile distilled water and also in a buffer solution: potassium acid phthalate- sodium hydroxide, with a pH after autoclaving of 5.2. All media were

[1] Read at the 25th General Meeting of the International Association for Dental Research, Chicago, Illinois, June 21, 1947. Received for publication July 17, 1947.

[2] This work was aided in part by a grant from the Procter and Gamble Company.

[3] Difco.

[4] Difco.

sterilized by autoclaving 12 minutes at 15 pounds pressure in tubes of measured liquid amounts.

In liquid, the inoculum was 0.1 ml. of a 1:10 to 1:1,000,000 dilution of a 48 hour broth culture. Incubation was at 37° C. for a maximum of 84 hours. Determinations of the activity of the agents were made by pH determinations (Beckman); turbidity, visual and photoelectric colorimetric (Evelyn); plate counts; and microscopic examination. The agents were added in dilutions of 1:10 to 1:2,000,000 either before or after autoclaving. When addition was after autoclaving, sterile agents were added by aseptic technique.

A total of 83 surface-active agents were tested. Classified on the basis of their mode of action, they were wetting, penetrating, dispersing, emulsifying and foaming agents, or presented combinations of two or more of these properties. On the basis of chemical activity, the agents were anionic, cationic and non-ionic. In this paper the term "agents" is used to indicate surface-active substances or products containing such a substance as one of their chief ingredients.

RESULTS

Table I gives the average results of 23 surface-active agents in producing inhibition or stimulation of the growth of a strain of oral *Lactobacillus*, by the Oxford cup method. All the agents listed completely inhibited in the 1:10 dilution with the exception of Tergavon C, Victawet B, Teel, and Artic Syntex 'M', which only partially inhibited in this dilution in 24 hours. There was little or no change in these findings at the end of 48 hours with the exception of Drene which inhibited the organisms at 24 hours completely, but by 48 hours only partial inhibition was observed.

Onyx (alkyl dimethyl benzyl ammonium chloride) demonstrates the activity of the inhibitory agents. There is inhibition complete to partial for 35 mm. with media change adjacent to cup. Note the area of stimulation beyond the inhibitory zone (*fig. 1*). Results are essentially the same at 48 hours.

At the 1:100 dilution 12 agents are listed showing complete to partial inhibition of the test organism with areas of diffusion varying from 9–40 mm. Three agents stimulated growth in this dilution: Michelene DCA beyond the inhibited area, Teel stimulation only and Tween 60 stimulation immediately around the porcelain cylinder with inhibition in the outer zone of diffusion. In the 10^3 dilution the activity of the agents was observed to be about the same as at the 10^1 and 10^2 dilutions, with a reduced area of diffusion and greater stimulatory activity appearing in the case of Ciba 'M', Soltex and Michelene DCA.

Resolin B and Glyco-trigamine were observed to inhibit the strain of oral *Lactobacillus* in dilutions of 10^5, $5x10^5$, and 10^6. Many of the remaining agents listed stimulated the organism to greater growth than was seen in the control plates.

Of the 83 agents tested in broth for stimulatory effect, 14 have produced significant results as observed by changes in density, bacterial counts, and pH of the medium. Changes in density were interpreted by amount of dispersion of

TABLE I

Activity of surface-active agents (on agar)

NAME	TYPE	DILUTION													
		10^1		10^2		10^3		10^4		10^5		5×10^5		10^6	
		Hours													
		24	48	24	48	24	48	24	48	24	48	24	48	24	48
Laurel Super sulfates	anionic	c20	c30			ca	ca			cas	cas			s	s
Tergavon 'C'	anionic	p33[1]	p35[1]			p18	p23			s++	s			s++	s
Onyx (73)	cationic	c35p40 s++45[2]	c35p40 s++45[2]	c25ps	c25p	c15		ca		sca		s14		s++10	
Ciba 'M'		c20p24		c18p25		s14	s++	sa		s++	s+/--			sa	s+/−
Vietawet 35 B	anionic	c40		c40		c18p		p20		pa		s		s++	
Soltex		c15	c18	c18p25		cas	ca	sa		s	s+/-	s++		s++	s
Ahcowet ANS	anionic	c30	c30			c12	ca			n	n			s	s
Aerosol 18	anionic	c18	c20			c10	p18			s	n			n	n
Ultrawet E		c40	c40	c35	c26p	p30	ca	p15	pas	s+	s	s	s	ps20	s
Resolin B		c40s				p30s				p20s				cp20s+	
Penetrant 1000	anionic	c40		c38		c25		pa		pa		s		s	
Michelene DCA		c20s30	cas++	cas18		s14	s+	sa		s15	s	s		s	s
Vietawet 54 B	non-ionic	cap15s++				cap15s				cas				cas	
Drene	anionic	c25p30	p40	c20p30		cas	p35	cas		s+++	s+++	s		s++	s++
Halo	anionic	c30				cp				s				s	
Teel	anionic	cap12		s		s		s		s		s		s	
Orvus	anionic	c40				c30				pas16				n	
Stablex G	anionic	c50				c25				pas				s++	
Stablex B	anionic	c25				ca				s++		s		s	
Tween 60	non-ionic	s18c25	s18c25	s10c20	s10c20	c12s	c12s	p14s	p16	cas	pas	pas	s	s	s
Petromix		c30p				c15p				pas13				pas18	
Artic Syntex M		cap20	p18	cap		caps	c10ps	caps		pas	pas++	pas++	s	pas++	s
Glyco trigamine		c20p				c17p				c15p				c14p	

c, complete inhibition in delimited area; p, partial inhibition; s, stimulation; n, no significant change.

+, ++, +++, ++++, diffuse zone of stimulation not measureable.

Numbers indicate diameter of delimited zone of inhibition or stimulation.

Dilution indicates concentration of agent in sterile distilled water in the cup.

1. Stimulation under and immediately around cup.
2. Media change adjacent to cup.

growth throughout the medium rather than by extent of growth at bottom and up the sides of the culture tube. The findings were observed using dextrose-special broth, pH 5.2,[5] and comparing with controls not containing surface-active agents.

Of the 14 agents found to produce this desired effect only one will be presented in detail in this preliminary report. Penetrant 1000, sodium alkyl naphthalene sulfate, an anionic surface active agent, in a dilution of 10^5 caused the strain of oral *Lactobacillus* to grow more rapidly than the same organism in broth alone. The culture tubes were observed to have a dispersed growth readily visible by inspection. Acceleration of growth was also measured by diminution of light transmission in a photoelectric colorimeter.[6] Absorption test tubes containing 20 ml. of broth-agent (dilution 10^5) mixtures were used. No significant changes

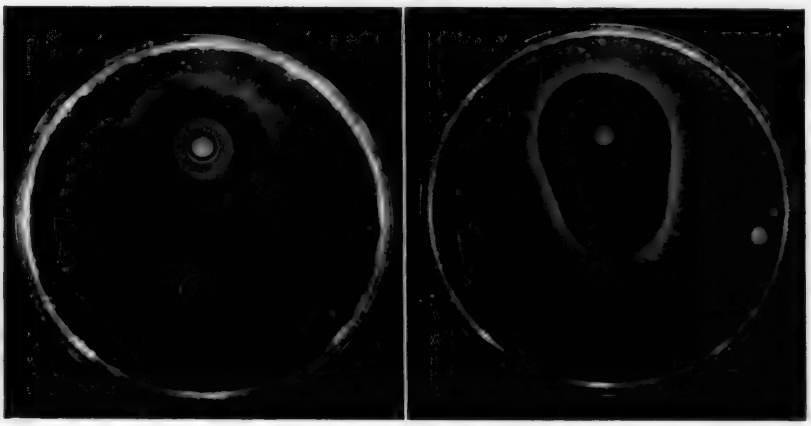

Fig. 1. A (left). Onyx—24 hour incubation. B (right). Onyx—48 hour incubation. Top—Experimental. Bottom—Control.

were observed in readings in the first 12 hours between test and control tubes. Slight density changes were observed in the second 12 hour period, with the agent-broth tubes allowing less light transmission. Acceleration of growth or dispersion of the organisms produced a galvanometric difference of 4 or greater. This difference in light transmission increased to 7 points at 72 and 84 hours. The general character of the growth curves, control broth and agent-broth, were similar. Greater acid production, indicating greater growth in comparison with the controls, was found to take place during the 12–24 hour period. The greatest pH difference was 0.2, a difference which persisted after the first 36 hours incubation.

COMMENT

Many surface-active agents appear to affect oral *Lactobacilli* by inhibiting their growth when used in lower dilutions and stimulating their growth in higher

[5] Difco.

[6] Evelyn Photoelectric Colorimeter, Filter 540.

dilutions. The exact mechanism of these actions is not known since it was not in the scope of the preliminary work to determine the specific physical and chemical reactivity of the agents.

During the course of these studies an attempt was made to determine changes in control and test-agent broth cultures by surface tension readings, using a tensiometer. However, the changes recorded were minute in all cases, and within the limits of statistical error.

Numerous bacterial counts have been made with a standard inoculum in agar to which surface-active agents had been added in specific dilutions. The results indicate in many cases that *Lactobacilli* will grow out in 18 hours in the presence of certain wetting agents, while in the control plate, colonies are not visible until 48 hours. In most cases a slight differential in the number of colonies in favor of the test media remained at the end of a 72 hour period.

While the problem of stimulation of growth is of interest to relatively few, in he opinion of the authors the inhibitory effects of these surface-active agents definitely indicate a potentiality in the field of caries-control in dentistry to be reported in subsequent papers.

SUMMARY

The effect of the addition of various surface-active agents on cultures of oral Lactobacilli has been investigated.

Stimulation and inhibition are demonstrated upon the addition of varied amounts of the agents in liquid and solid media.

An indication of the potentialities of these findings is noted.

The authors wish to acknowedge with gratitude the many helpful suggestions and donation of the cultures of oral *Lactobacilli* from Dr. T. J. Hill, Professor of Oral Pathology, Western Reserve University, School of Dentistry.

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TRYPTOPHANE IN CARBOHYDRATE METABOLISM[1]

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Greater oxidative capacity toward starch in the saliva of persons with rampant caries and greater reductive capacity toward starch in the saliva of persons free from caries has been reported by Turner and Crowell (1). The present paper presents an extension of these studies and includes a report of the titratable acidity developed in saliva-starch mixtures incubated together at room temperature for a period of 2 hours.

In the studies of titratable acidity, the acid developed at zero time is subtracted from the acid developed at 120 minutes to secure the reading of acid produced in the two-hour interval. The saliva of individuals with rampant active caries develops, in this interval, about 8 times as much acid as that of individuals who are free from dental caries. The measurement of acid is with .01 N NaOH and with phenolphthalein as indicator. Table I gives individual readings and averages for the 2 groups. The individuals reported as rampant caries not only show extensive but also active caries.

Among the individuals showing rampant caries were 8 young adults, ages 18–19, who had had between 7 and 10 fillings or cavities each in the previous 6 months. Four of these individuals volunteered to ingest, for a period of 3 months, crystalline tryptophane in small doses adjusted to body weight. The other 4 were controls. A physician's examination of the volunteers and laboratory tests of urine, saliva, and blood preceded and followed the tryptophane ingestions. Two of these individuals showed a drop (see Table II) in acid production during the ingestion of tryptophane. M. P. who did not follow this trend had had poliomyelitis in the fall of 1945. In spite of taking more tryptophane than the others, her saliva did not show the tryptophane level associated with freedom from caries until the very end of the ingestion period. She developed further caries during the 3-month period. P. P. is her twin sister. The acid production of the test subjects and the 4 carious controls who did not ingest tryptophane is reported in Table II.

Accompanying the low acid production from starch by the salivas of caries-free individuals, there was a high production of reducing substance as indicated with "Clinitest"[2] procedure and as measured by reduction of preformed starch blue. The caries-free group on the average produced .309% of reducing substance from saliva starch in a two-hour incubation at room temperature. The

[1] Received for publication July 23, 1947.

[2] It is recognized that the "Clinitest" procedure is a rough measure of reducing substance, but it was used because of the simplicity of its use in the clinic and in order to get an indication of the trend.

TABLE I

Acid developed per cc. in 2-hour period at room temperature from equal parts of saliva and 1% corn starch solution as measured by .01 N NaOH to neutralize

RAMPANT CARIES		CARIES FREE	
E. C.	.5 cc.	.1 cc.	B. McC.
P. N.	.35	.1	V. R.
G. S.	.3	.0	C. C.
R. McC.	.4	.0	C. G.
H. McC.	.5	.0	P. L.
M. T.	.35	.0	C. L.
C. DeD.	.3	.1	S. W.
M. H.	.3	.0	D. L. C.
E. G.	.35	.05	D. M. C.
J. B.	.35	.2	Jo. C.*
J. W.	.1	.05	Je. C.
E. B.	.6	.0	T. L. F.
E. S.	.2	.0	W. B.
M. L.	.35	.05	E. E.
L. T.	.35	.05	J. F.
P. P.	.1	.0	L. C.
M. P.	.1	.0	F. A.
Av. =	.323 cc.	Av. =	.04 cc.

* Glandular infection.

TABLE II

Acid developed per cc. in 2-hour period at room temperature from equal parts of saliva and 1% corn starch solution as measured by .01 N NaOH to neutralize

	BEFORE TRYPTOPHANE	AFTER 3 MONTHS ON CRYSTALLINE TRYPTOPHANE
Test group		
L. T.	.35 cc.	.0 cc.
M. P.	.1	.2
P. P.	.1	.1
M. L.	.35	.05
Av.	.225 cc.	.088 cc.
Carious controls		
		NO TRYPTOPHANE INGESTIONS
J. B.	.35 cc.	.35 cc.
J. W.	.1	.3
E. B.	.6	.65
E. G.	.35	.2
Av.	.35 cc.	.375 cc.

rampant-caries group produced .163% of reducing substance under like conditions. The test group, before tryptophane ingestion, produced on the average

.094% of reducing substance from saliva-starch in a two-hour incubation period at room temperature. After the three-month period of tryptophane ingestions the test group produced on the average .312% of reducing substance under like conditions. The carious controls tested at the same time produced .083% of reducing substance.

It would appear that accompanying the lessening of acid production from saliva and starch after tryptophane ingestion there is also an increase in the amount of reducing substance. The contrasts in the production of acid and reducing substance from the rampant-caries, caries-free, and test groups are shown in Table III.

Table IV shows the experience of the Test Group vs. the Controls in decolorization of a preformed starch blue dye stuff. This decolorization is essentially a reduction process. The acceleration of the reduction process is shown a shorter period for decolorization.

TABLE III

Contrasts of averages in the production of acid and of reducing substance

	RAMPANT-CARIES GROUP OF TABLE I	CARIES-FREE GROUP OF TABLE I	TEST GROUP (SEE TABLE II)	
			Before tryptophane ingestion	After 3 months of tryptophane
Acid per cc. out of saliva-starch in 2-hour incubation as measured with .01 N NaOH.....	.323 cc.	.04 cc.	.225 cc.	.088 cc.
Reducing substance from saliva-starch 2-hour incubation "Clinitest".....	.163%	.309%	.094%	.312%

While the evidence from this pilot study is insufficient for drawing definite conclusions concerning the effect of tryptophane as a factor in the prevention of caries, the fact that, except for M. P., whose case has been described, the 4 girls of the pioneer group showed no new cavities while the 4 girls in the carious control group showed a total of 11 new cavities, is suggestive of beneficial results.

There was no significiant change in blood pressure, in body weight, or in redoxpoise drop. Dextrinizing time was lengthened for the experimental group. Their blood sugar drop was held to an average drop of 9 points by adjusting the frequency of ingestion. Considerable caution was used to keep the dosage of tryptophane sufficiently low to avoid possible hypoglycemia and yet maintain a salivary level comparable with that found among individuals free from tooth decay.

Recent reports from 2 groups of workers help to interpret something of the mechanism by which tryptophane may function in carbohydrate metabolism. Fraenkel-Conrat, Brandon and Olcott (2) have shown that the tryptophane of gramicidin condenses with aldehydes. It is interesting to note in this connection the fact that chemical color tests for tryptophane commonly depend on condensation with aldehydes (formaldehyde, benzaldehyde, glyoxylic acid, etc.). One might assume that condensation of tryptophane with the aldehyde would lead to at least temporary denial of the aldehyde for oxidation to acid.

Condensation with glucose through its aldehyde may likewise account for the blood sugar drop consistently noted by us during tryptophane ingestions (3). That this represents a tendency to storage (as in hyperinsulinism) but does not prevent calling on glycogen reserves is shown by glucose tolerance readings for two individuals following tryptophane ingestions. These readings stay down at the half-hour and hour intervals but rise at 2 hours.

The second study of interest in helping to explain the lessening of acid production by the action of salivas on starch following tryptophane ingestion is the influence of sodium bisulphite on acid production in saliva (4). This study reports lessening of acid production in saliva in vitro when aldehyde is bound

TABLE IV

Decolorization of preformed starch blue pH7 redox + 310

	BEFORE TRYPTOPHANE	AFTER 3 MONTHS ON TRYPTOPHANE INGESTIONS
Test group		
L. T.	31 min.	5 min.
M. P.	22 "	4 "
P. P.	54 "	6 "
M. L.	60 "	5 "
Av.	42 min.	5 min.
Controls		
J. B.	25 min.	4 min.*
J. W.	22 "	32 "
E. B.	17 "	12 "
E. G.	70 "	75 "
Av.	33.5 min.	31 min.

* Three local applications of NaF during 3-month period; it is interesting to note that this individual's acid production remained at .35 cc.

by sodium bisulphite. One might hypothesize that tryptophane or a similar indole-containing molecule, as part of an enzyme system in the saliva, leads to a lessening of acid out of starch by condensation with aldehydes during starch degradation.

In the course of microscopic examination of crystalline forms from saliva, tyrosine was found. After the hydrolysis of saliva with H_2SO_4 (12cc H_2SO_4; 88cc H_2O), it was possible to identify tyrosine sheaves microscopically as a common and abundant constituent. Chemical confirmation of this microscopic finding was readily obtained through Millon's reagent. Hexagonal crystals which may be cystine also appear on the slides from saliva and from Beta amylase when examined under a petrographic microscope. (For acid hydrolysis of saliva in the search for cystine crystals, HCl may be preferred to H_2SO_4.)

As in other phenols, substitution of halogens for hydrogen in tyrosine takes place readily, yielding the corresponding dihalogenated tyrosine (5). Di-iodo-tyrosine is an essential constituent of the secretion of the thyroid gland, which is associated with carbohydrate metabolism. One might speculate, therefore, that the well-known enzyme inhibitory action of fluorides (6) may depend upon the formation of a di-fluoro-tyrosine which might not have the catalytic action in carbohydrate metabolism possessed by di-iodo-tyrosine.

We acknowledge with gratitude the medical supervision of the Experimental Group by Priscilla White, M.D.

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PENICILLIN AND CARIES ACTIVITY[1]

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In studying the inhibitory effects of various agents on carbohydrate fermentation in saliva, penicillin appeared far more effective than other substances tested. In addition to in vitro and in vivo tests, animal experiments were carried out to determine the effect of penicillin on dental caries.

IN VITRO STUDIES

To test for interference with carbohydrate fermentation, the following procedure was used: 4 cc. of pooled stimulated saliva added to 1 cc. of a 20% glucose solution were incubated at 37° C. for 4 hours. At the end of this period, the pH of the saliva-glucose mixtures was determined electrometrically. As a control, 4 cc. of the saliva were incubated with 1 cc. of distilled water. The test agents were added in various concentrations to the saliva-glucose mixtures before incubation.

Results typical of 20 tests are shown in Table I. Pooled saliva and glucose alone produces a pH of 4.0 after 4 hours incubation. However, when 10 units of penicillin are present, no acid is formed.

IN VIVO STUDIES

To determine penicillin activity in the human mouth and how long such an effect would last, the following tests were conducted: To establish control values 1 cc. of 20% glucose was added to 4 cc. of saliva collected from each of 4 students, and the samples incubated for 4 hours. After the incubation period the pH was determined. Then each student rinsed his mouth with 20 cc. of distilled water containing 1000 units penicillin. Immediately thereafter, 30 minutes, 1 hour, and 2 hours later another 4 cc. sample of saliva was obtained from each of the subjects, incubated with glucose, and the pH determined as described above. The results are shown in Table II. The pH of the control samples ranged from 4.0 to 5.1; whereas those samples taken as late as 2 hours after the penicillin rinse were not depressed below pH 6.8 to 7.2. To eliminate the possibility that mouthwashing followed by repeated stimulation and spitting saliva might of itself be responsible for the reduced capacity of the saliva to form acid, additional controls were run. Table III shows the results of rinsing with distilled water alone. It is obvious then that the repeated spitting did not materially effect acid production by saliva. These findings were consistent in 40 tests and

[1] Read at the 25th General Meeting of the International Associtaion for Dental Research, Chicago, June 21–22, 1947. Received for publication July 23, 1947.

indicated that penicillin is effective in preventing acid formation in the mouth for a considerable period.

TABLE I

Acid production in saliva-penicillin-glucose mixtures

	pH AFTER INCUBATION			
	1 hr.	2 hrs.	3 hrs.	4 hrs.
4 cc. saliva + 1 cc. 20% glucose	6.5	5.0	4.5	4.0
4 cc. saliva + 1 cc. H_2O	7.0	6.9	7.0	7.0
4 cc. saliva + 1 cc. 20% glucose + 10 units penicillin	6.8	6.8	6.8	7.0
4 cc. saliva + 1 cc. 20% glucose + 50 units penicillin	6.8	6.8	7.0	7.0
4 cc. saliva + 1 cc. 20% glucose + 100 units penicillin	6.8	7.0	7.0	7.2
4 cc. saliva + 1 cc. 20% glucose + 500 units penicillin	7.0	7.0	7.0	7.2
4 cc. saliva + 1 cc. 20% glucose + 1000 units penicillin	7.0	7.0	7.0	7.2
4 cc. saliva + 1 cc. 20% glucose + 10,000 units penicillin	7.0	7.0	7.0	7.2

TABLE II

*pH of saliva-glucose mixtures after penicillin mouth wash**

	pH AFTER 4-HR. INCUBATION			
	Subject A	Subject B	Subject C	Subject D
4 cc. saliva before mouth rinse + 1 cc. 20% glucose	4.0	5.1	4.7	4.6
4 cc. saliva imm. after pen. rinse + 1 cc. 20% glucose	5.5	7.0	6.8	6.8
4 cc. saliva ½ hr. after pen. rinse + 1 cc. 20% glucose	6.6	7.2	7.0	7.0
4 cc. saliva 1 hr. after pen. rinse + 1 cc. 20% glucose	6.6	7.1	7.0	7.0
4 cc. saliva 2 hrs. after pen. rinse + 1 cc. 20% glucose	6.8	7.2	7.0	7.0

* 20 cc. of H_2O containing 1000 units of penicillin.

ANIMAL EXPERIMENTS

Animal experiments were carried out to determine whether the inhibitory effect of penicillin on carbohydrate fermentation in saliva actually reduced dental caries.

Twenty-four male golden Syrian hamsters were put on a caries-producing diet[2] at weaning age. They were divided into 3 groups of 8 animals. Group I

[2] 56% corn starch.
30% powdered whole milk;

had their teeth brushed once daily with a commercial dentifrice. Group II had their teeth brushed daily with a penicillin-containing dentifrice (1000 units of penicillin per gram added to dentifrice use in Group I). Group III did not have their teeth brushed, but had the same diet as Group I and II. During the course of the experiment one animal from Group II was killed accidentally. All animals

TABLE III

*pH of saliva-glucose mixtures after mouth wash**

	pH AFTER 4-HR. INCUBATION			
	Subject A	Subject B	Subject C	Subject D
4 cc. saliva before mouth rinse + 1 cc. 20% glucose	4.5	4.4	4.4	4.6
4 cc. salive imm. after mouth rinse + 1 cc. 20% glucose	5.4	4.6	4.9	4.8
4 cc. saliva 1 hr. after mouth rinse + 1 cc. 20% glucose	4.8	4.7	4.7	4.8
4 cc. saliva 2 hrs. after mouth rinse + 1 cc. 20% glucose	4.7	4.7	4.8	4.8

* 20 cc. of H_2O.

TABLE IV

Influence of a penicillin-containing dentrifrice on caries experience of hamsters

ANIMAL NO.	GROUP I CONTROL TOOTHPASTE ANIMALS			GROUP II PENICILLIN TOOTHPASTE ANIMALS			GROUP III CONTROL ANIMALS		
	Unit area affected	# Cavities	# Carious teeth	Unit area affected	# Cavities	# Carious teeth	Unit area affected	# Cavities	# Carious teeth
1	75	3	1	0	0	0	4,893	43	12
2	645	10	6	0	0	0	6,031	26	12
3	811	10	8	0	0	0	1,583	16	9
4	203	2	1	534	5	5	5,428	18	10
5	305	6	4	0	0	0	5,480	20	12
6	412	7	4	0	0	0	1,396	17	7
7	1,090	7	6	142	1	1	200	6	4
8	90	2	1				1,046	6	5
Average....	454	5.87	3.87	97	0.86	0.86	3,257	19.0	8.87

were sacrificed on the thirty-fifth day of the experiment and their jaws prepared for examination.

The carious areas were counted for each animal under a dissecting microscope and recorded on the chart described by Keyes (1). The results in Table IV are tabulated according to the number of cavities in each animal, the number

10% powdered sugar.
3% alfalfa.
1% NaCl.

of carious teeth, and the surface area involved[3] by the caries process. The difference in caries experience of the penicillin group when compared to the other groups is marked, the average number of cavities in the penicillin group being 0.86 as compared with 19 in the control group. In only one animal did penicillin fail to materially reduce the incidence of caries. Five of the 7 animals in the penicillin group were completely caries free.

COMMENT

The results of these animal experiments are in complete accord with those of McClure (2), who found that small amounts of penicillin added to the diet of cotton rats inhibited dental caries.

Our finding that penicillin interferes with carbohydrate fermentation by saliva in vivo and in vitro, and that it prevents caries in hamsters, suggests that this agent might have value in combatting human dental caries. As far as is known penicillin has no effect either on dental structures or on mammalian metabolism and its activity is purely antibacterial. Nevertheless, this agent actively prevents caries in animals.

The only logical conclusion is that this caries-inhibiting effect is a result of antibacterial activity and/or interference with carbohydrate fermentation. This is good evidence that these factors are important in the causation of caries.

SUMMARY

Minute amounts of penicillin inhibit acid formation in saliva-carbohydrate mixtures.

The capacity of saliva to ferment carbohydrates is eliminated for several hours following the use of a penicillin mouth wash.

Brushing penicillin mixtures on hamsters' teeth virtually prevents dental caries.

The above findings offer further evidence that the fermentation of carbohydrates by oral bacteria is important in the etiology of dental caries.

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[3] The figures for surface area involved are devised by superimposing millimeter paper on the chart and then counting the number of square millimeters of areas involved.

EFFECTS OF TOPICAL SODIUM FLUORIDE IN THE MANDIBULAR TEETH[1]

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One of the puzzles in the study of topically-applied sodium fluoride as a caries-preventive agent has been the apparent relative ineffectiveness of the chemical when applied to the mandibular teeth. While field studies have shown uniformly significant reductions in maxillary caries, Bibby reports only slight and chance reduction in caries of the mandibular teeth following application of an aqueous solution of sodium fluoride (1), or following the use of a fluoride-pumice prophylaxis mixture (2). In a study reported by Jordan, Wood, Allison and Irwin there is a higher incidence of caries in the untreated than in the treated mandibular quadrants following 2 applications of a 2% aqueous solution (3).

Bibby has suggested that this may be due to a leakage of the fluoride solution during treatment from an incompletely-isolated lower quadrant to the opposite side, with consequent partial protection of the control teeth (4).

On the other hand, Knutson and Armstrong have observed a definite and significant caries reduction in the mandibular teeth of Minnesota children given from 7 to 15 treatments with a 2% aqueous solution, the protection extending through the third study year (5, 6, 7). The Knutson-Armstrong series in particular is reported in sufficient detail to permit the application of some of the methods ordinarily used in the epidemiology of communicable disease, and to permit estimations of sampling error. Particularly useful in such an analysis is the caries attack rate, which expresses the percentage of teeth free from caries at the beginning of a study which became carious during the period of observation. When the latest of the Knutson-Armstrong series (8) is further analyzed new light is shed upon the questions of the effectiveness of topical sodium fluoride in the mandibular teeth.

In the original Knutson-Armstrong study a comparable group of wholly untreated children was observed for 2 years in order that the opposite-quadrant method of control might be tested. In these children the 2 year caries attack rates in the mandibular teeth were 9.4 and 9.3 in the left and right quadrants, respectively, compared with an attack rate of 9.3 in the untreated right mandibular quadrants of the study children. In this study at least, there is no indication of a leakage of treatment solution to the control side as postulated by Bibby. Bilateral symmetry was observed in the caries incidence of the untreated children. It was concluded that opposite-quadrant controls were adequate and the untreated control group was discarded in subsequent studies.

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The latest of the Knutson-Armstrong studies (8) reports upon 1,458 children of 7 grade schools and 2 junior high schools of Rochester, Minnesota. The children were divided into three groups having approximately the same age-sex distribution, and were given 2, 4, or 6 applications of 2% topical sodium fluoride to one quadrant of each jaw, with the opposite quadrant left untreated as a control. The children did not receive a dental prophylaxis at the beginning of the treatment series. Results are reported after 2 years.

Attack rates in the treated maxillary teeth are 16.8, 13.5, and 12.9, and in the treated mandibular teeth 9.3, 7.6, and 6.3 respectively, after 2, 4, and 6 applica-

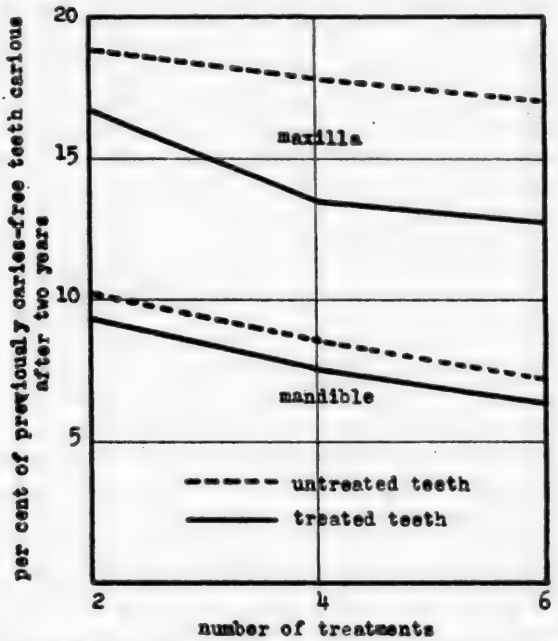

Fig. 1. Comparison of caries attack rates in treated and untreated mouth quadrants of 1458 children given 2, 4 or 6 topical fluoride treatments without a prior prophylaxis. (After Knutson, Armstrong and Feldman.)

tions of the chemical. A similar decline in the incidence of dental caries is seen in the untreated quadrants, where the maxillary teeth show rates of 18.8, 17.9, and 17.2 and the mandibular teeth rates of 10.3, 8.7, and 7.2 respectively after 2, 4, and 6 treatments to the opposite side. It would seem that the fluoride treatment given the opposite quadrant has conferred some resistance to caries to the presumably untreated teeth which were utilized as controls, and that this effect is more pronounced in the mandibular than in the maxillary teeth.

This result is shown graphically in *fig. 1*.

Statistical analysis suggests that the results in the maxillary quadrants, as shown in *fig. 1*, are probably chance. While the coefficient of correlation be-

tween the attack rates in the untreated maxillary quadrants and the number of treatments given the opposite side is high, $-.9963 \pm .0138$, the variation from the mean rate of 17.96 is small, and the chi square test yields a P of .50. In a personal communication Knutson stated that the coefficient of correlation drops to $-.2526 \pm 0.013$ when the analysis is made on the basis of individual age classes (9).

TABLE I

Dental caries experience in the untreated quadrants of the maxillary teeth of 1,458 Minnesota children given 2, 4, or 6 treatments with sodium fluoride in the opposite quadrants (Knutson)

NUMBER OF TREATMENTS IN OPPOSITE QUADRANT	NUMBER OF CARIOUS TEETH					NUMBER OF TEETH NOT CARIOUS					ATTACK RATE	NUMBER OF TEETH
	O	E	O - E	$(O-E)^2$	$\frac{(O-E)^2}{E}$	O	E	O - E	$(O-E)^2$	$\frac{(O-E)^2}{E}$		
2	316	302	14	196	.649	1368	1382	−14	196	.142	18.7648	1684
4	324	325	−1	1	.003	1486	1485	1	1	.000	17.9005	1810
6	300	312	−12	144	.462	1440	1428	12	144	.101	17.2414	1740
	940	939			1.114	4294	4295			.243	17.9595	5234

$n = 2$. $\chi^2 = 1.357$. $P =$ approx. .50.

TABLE II

Dental caries experience in the untreated quadrants of the mandibular teeth of 1,459 Minnesota children given 2, 4, or 6 treatments with sodium fluoride in the opposite quadrants (Knutson)

NUMBER OF TREATMENTS	NUMBER OF TEETH	NUMBER OF CARIOUS TEETH					NUMBER OF TEETH NOT CARIOUS					ATTACK RATE
		O	E	O - E	$(O-E)^2$	$\frac{(O-E)^2}{E}$	O	E	O - E	$(O-E)^2$	$\frac{(O-E)^2}{E}$	
2	1,920	198	168	30	900	5.357	1722	1752	−30	900	.514	10.3125
4	2,039	178	178	0	0	0.0	1861	1861	0	0	.000	8.7298
6	1,947	141	170	−29	841	4.947	1806	1777	29	841	.473	7.2419
	5,906	517	516			10.304	5389	5390			.987	8.7538

$n = 2$. $\chi^2 = 11.291$. $P =$ approximately .005.

In the mandibular quadrants, on the other hand, the chi square test establishes odds of approximately 200 to 1 that the variation from the mean attack rate of 8.75 is not due to chance. The coefficient of correlation between attack rates in the untreated quadrants and the number of treatments given the opposite side, using lumped age-group classes as pictured in *fig. 1*, is $-.9998 \pm 0.013$. When age-groups are analyzed separately, the coefficient of correlation is $-.545 \pm 0.013$ (9).

It appears, then, that the caries attack rate in the untreated mandibular

teeth in this study drops in relation to the number of treatments given the opposite quadrant, and that this drop occurs as a result of the treatment, since all other variables had been excluded. No such phenomenon can be discovered in

TABLE III

Correlation of caries attack rates in untreated quadrants (Knutson) with number of treatments given opposite quadrants—maxillary teeth

NUMBER OF TEETH	NUMBER OF TREATMENTS			CARIES ATTACK RATE			PRODUCT MEASURES
	O_x	d_x	d_x^2	O_y	d_y	d_y^2	O_xO_y
1,684	2	−2	4	18.7648	.7959	.6335	37.5296
1,810	4	0	0	17.9005	−.0684	.0047	71.6020
1,740	6	2	4	17.2414	−.7275	.5293	103.4484
		−2			−.7959		
5,234	12	2	8	53.9067	.7959	1.1675	212.5800

$$M_x = \frac{12}{3} = 4 \qquad M_y = \frac{53.9067}{3} = 17.9689$$

$$\sigma_x = \sqrt{\frac{8}{3}} = \pm 1.634 \qquad \sigma_y = \sqrt{\frac{1.1675}{3}} = \pm .624$$

$$r = \frac{\frac{212.58}{3} - (4 \times 17.9689)}{1.634 \times .624}$$

$$= \frac{70.86 - 71.8756}{1.0196}$$

$$= \frac{-1.0156}{1.0196}$$

$$r = -.9961$$

$$SE_r = \frac{1}{\sqrt{N}} = \frac{1}{\sqrt{5234}} = \frac{1}{72.35} = \pm .0138$$

Regression equation:

$$y - 17.9689 = -.9961 \frac{.624}{1.634}(x - 4)$$

$$y - 17.9689 = -.9961 \times .3819\ (x - 4)$$

$$y - 17.9689 = -.3804\ (x - 4)$$

$$y - 17.9689 = 1.5216 - .3804x$$

$$y = 19.4905 - .3804x$$

Therefore, when

$x = 0$, $y = 19.4905$

$x = 2$, $y = 18.7297$

$x = 4$, $y = 17.9689$

$x = 6$, $y = 17.2081$

the earlier reports (5, 6, 7), in which comparable children were studied by the same investigators, using the same methods except for the inclusion of a prior prophylaxis.

It follows that a somewhat greater reduction in caries incidence was achieved

TABLE IV

Correlation of caries attack rates in untreated quadrants (Knutson) with number of Treatments given opposite quadrants—mandibular teeth

NUMBER OF TEETH	NUMBER OF TREATMENTS			CARIES ATTACK RATE			PRODUCT MEASURES
	O_x	d_x	d_x^2	O_y	d_y	d_y^2	O_xO_y
1,920	2	−2	4	10.3125	1.5511	2.4059	20.6250
2,039	4	0	0	8.7298	−.0316	.0010	34.9192
1,947	6	2	4	7.2419	−1.5195	2.3089	43.4514
		−2			−1.5511		
5,906	12	2	8	26.2842	1.5511	4.7158	98.9956

$$M_x = \frac{12}{3} = 4 \qquad M_y = \frac{26.2842}{3} = 8.7614$$

$$\sigma_x = \sqrt{\frac{8}{3}} = \pm 1.634 \qquad \sigma_y = \sqrt{\frac{4.7158}{3}} = \pm 1.253$$

$$r = \frac{\frac{98.9956}{3} - (4 \times 8.7614)}{1.634 \times 1.253}$$

$$= \frac{32.9985 - 35.0456}{2.0474}$$

Formula:

$$= \frac{-2.0471}{2.0474} \qquad r_{xy} = \frac{\frac{\Sigma O_x O_y}{N} - M_x M_y}{\sigma x \, \sigma y}$$

$$r = -.998534$$

$$SE_r = \frac{1}{\sqrt{N}} = \frac{1}{\sqrt{5906}} = \frac{1}{76.85} = \pm .01301$$

Regression equation:

$$(y - M_y) = r \frac{\sigma y}{\sigma x} (x - M_x)$$

$$y - 8.7614 = -.998534 \frac{1.253}{1.634} (x - 4)$$

$$y - 8.7614 = -.998534 \times .7688 \; (x - 4)$$
$$y - 8.7614 = -.7657 \; (x - 4)$$
$$y - 8.7614 = 3.0628 - .7657x$$
$$y = 11.8242 - .7657x$$

Therefore, when
$x = 0$, $y = 11.8242$
$x = 2$, $y = 10.2928$
$x = 4$, $y = 8.7614$
$x = 6$, $y = 7.2300$

in the mandibular teeth than that estimated on the basis of the biased results in the untreated quadrants. Solution of the regression equation gives $y = 11.8242 - .766x$, suggesting that the attack rate would have been in the neighborhood

of 11.8 in the mandibular teeth of wholly untreated but otherwise comparable children. On this basis the caries reduction in the mandibular quadrants following 6 treatments was approximately 46.6%. If it is assumed that 2 treatments did not affect the untreated side and that the attack rate noted in the control quadrants of these children represents the true control, the reduction is 38.8%.

This method of analysis is not applicable to the other studies previously mentioned. In some of them insufficient data are furnished. In that reported by Jordan, Wood, Allison and Irwin the 3 groups of children lived in different communities where prevalence rates may have differed, and each group was observed by a different examiner. The analysis may, however, explain at least in part the disappointing results observed in the lower quadrants of an opposite-quadrant controlled study where a considerable length of time has elapsed between the initial prophylaxis and the final treatment in the series, or where the initial prophylaxis has been omitted.

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A DETERMINATION OF THE NUMBER OF SURFACES INVOLVED IN CARIOUS EXTRACTED TEETH[1]

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Considerable work in relation to the study of dental caries has been done using the designation, DMF (decayed, missing and filled permanent teeth), advocated by Klein, Palmer and Knutson (1). They mention an evaluation including tooth surfaces but assign no numerical value to a missing tooth. More recently, detailed studies have appeared using the designation, DMFTS (decayed, missing and filled tooth surfaces) (2, 3). In these reports, decayed and filled surfaces have been accurately tabulated, but the number of surfaces involved in a missing tooth has been arbitrarily assigned. A caries index proposed in 1931 by Bodecker (4) suggested that completely destroyed crowns of teeth with only roots remaining should count 5 points in the score of affected surfaces, and lost teeth, 3 points. Klein and Palmer (3) stated that if a one-surface involvement can affect the pulp and necessitate extraction of the tooth, such virulence could be considered equivalent to an attack on 5 surfaces. Accordingly, extracted teeth in their calculations were considered to have had five-surface involvements. While it is true that 5 surfaces are lost to the mouth under such conditions, it is evident that this value does not give a true picture of caries previously existent in missing teeth.

In this report, we wish to present the number of tooth surfaces found by extraoral inspection to have been carious in a series of extracted deciduous and permanent teeth.

PROCEDURE

The dental clinics of the State University of Iowa Children's Hospital and of the Pedodontic Department of the College of Dentistry, routinely save all teeth which are extracted, and file them according to patient number in a glycerin preservative solution in individual specimen bottles. The children whose teeth were studied come to the dental school from Iowa City and outlying territory. The hospital cases were drawn from the entire state. Middle class individuals, both ill and healthy, predominate. Each tooth considered in this report is supported by a detailed clinical examination according to the procedure by Boyd and Cheyne (5). At the present time, approximately 5,000 extracted teeth are in the files. All were considered in the course of this study. Only

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those which fell into the category of extractions due to caries, and according to the clinical record, were removed immediately after a diagnosis indicated such a course of action, were included. From this group, 475 were carefully examined to determine the number of surfaces involved. From the record of each patient, the age at time of extraction was determined, and if it was near the normal age for exfoliation, the tooth was discarded regardless of the amount of remaining root substance or carious involvement. A total of 86 teeth were thus rejected. Later, 11 teeth were rejected because of the inability to determine the patient's age with precision at the time of extraction. One tooth, likewise, was rejected because its loss was due primarily to trauma. The total number of teeth rejected from those originally included in the study was 98. The total number of teeth (both permanent and deciduous) included in the final analysis was 377.

For a number of years, the examiners in the pedodontic clinics have divided enamel lesions into 2 classes: namely, noncarious and carious. The term "noncarious" (NC) refers to suspicious areas found for the most part in the enamel of the tooth. Clinically, they are mere roughenings, with few or no loose enamel rods, and giving no penetrability to a sharp explorer tine. They are, in brief, areas not considered as truly carious, but rather, places to watch for further activity of the carious process. They are not considered in the totals presented in this paper, but are tabulated for reference by examiners who include such lesions under a similar system of caries classification.

The appearance of true enamel caries is familiar to most dental examiners. Usually these are described as those lesions which exhibit whiteness, or sufficient roughness that scratching of the area with an explorer tine noticeably dislodges remaining enamel rods. Lesions which are more advanced give a noticeable stick of the explorer tine in the enamel under moderate pressure, without involving the dentin. These lesions should be included with caries found in the dentin if a true evaluation of caries is sought.

In order to determine the ratio between the teeth with crowns totally destroyed and the roots subsequently extracted but not filed in specimen bottles, and teeth with some portion of the crown still intact, such as those studied, the following method was used: Charts were removed at random from the clinic files. The first 200 charts having a record of any extraction were retained. From these was calculated the ratio of root fragments to teeth indicated for extraction, but with some portion of the crown remaining. This method disclosed a ratio of 19 roots (10%) to 185 extractions of teeth with some portion of the crown remaining. Calculations of dentinal caries, therefore, include a 10% addition in order to correct for root fragments extracted following total crown destruction. By using such a method, teeth extracted after total crown destruction, and not filed, were considered in the calculations.

PRESENTATION OF MATERIAL

Tables I and II present the data on carious surfaces in extracted teeth according to sex, tooth type and age. No attempt has been made in this study to analyse the relative incidence of extractions in the various tooth and age groups.

The mean number of tooth surfaces involved with noncarious enamel lesions, enamel caries, and dentinal caries, is listed for each tooth type. Extracted teeth from boys happened to comprise a larger group than those from girls, the ratio

TABLE I

Carious surfaces in extracted teeth classified according to tooth type and age in males

TOOTH*	AGE														CARIOUS SURFACES			
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Enamel NC	Enamel C	Dentin**	Dentin enamel
1D			2	1	1	1	2	6	1						.308	.308	3.754	4.062
2D			2	4	4		6								.187	.062	3.988	4.050
3D					1												4.1	4.1
6D		1															4.1	4.1
8D							1								1.	1.	3.	4.
9D			2	2	7	2	4								.125	.063	4.047	4.110
10D				2	1	4	7	2							.063	.375	3.538	3.913
11D			2	3	6	4	4	2							.286	.333	3.843	4.176
12D		2	1	6	6	5	9								.069	.034	4.037	4.071
18D									1						1.		3.2	3.2
19D		1	2	2	5	3	10								.217	.043	4.061	4.104
20D			3	2	6	4	8	8							.129	.161	4.187	4.348
2																		
3										4		3			.288		4.229	4.229
5																		
8												1					4.1	4.1
9												2					4.55	4.55
10												2					4.1	4.1
12													1				5.	5.
14								2		2		2	2		.125	.125	4.1	4.225
15																		
18													1				5.	5.
19							1	2	3	5	3	2	4		.150	.3	4.055	4.355
23												1					5.	5.
24													1				4.1	4.1
29										1						1.	4.	5.
30						1	1	1	2	2	2	3	3	1		.25	4.269	4.519
31												1		1			4.55	4.55

* Permanent teeth are numbered consecutively from upper right third molar to upper left third molar, then from lower left to lower right third molar. A similar method is followed in numbering deciduous teeth.

** Calculations of dentinal caries includes a 10% addition in order to correct for root fragments extracted following total crown destruction.

being 233:144. A total of 104 permanent teeth were examined; 63 from males and 41 from females. A total of 273 deciduous teeth were examined; 170 from males and 103 from females. Of all the permanent teeth examined, 88 were first molars. Table III presents evaluated comparisons of the mean number of

surfaces involved with enamel and dentinal caries in each sex according to permanent and deciduous tooth groupings. The mean number of surfaces involved in the boys' permanent teeth is 4.378; in the boys' deciduous, 4.096; in the girls'

TABLE II

Carious surfaces in extracted teeth classified according to tooth type and age in females

TOOTH*	AGE														CARIOUS SURFACES			
															Enamel		Dentin**	Dentin Enamel
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	NC	C		
1D					1	4	4	4							.077	.231	3.961	4.192
2D				1		2	4								.286		4.357	4.357
3D		1				1	1										4.1	4.1
6D																		
8D																		
9D					2	1	2									.2	4.46	4.46
10D					2	4	4	3								.077	3.823	3.900
11D				5	2	6	4	4							.142	.381	3.5	3.881
12D	1		1	4	2	3	3								.071		3.714	3.714
18D				1													4.1	4.1
19D			1	3		3	2								.111		4.399	4.399
20D				4	1	1	7	4							.059	.176	4.206	4.382
2													1				5.	5.
3							1			2	1	1	1		.167		4.700	4.700
5												1					4.1	4.1
8																		
9																		
10																		
12																		
14							1			1							4.1	4.1
15														1			5.	5.
18														1			3.2	3.2
19							2	1	1	3	3	5	1		.063	.125	4.269	4.394
23																		
24																		
29																		
30							3		1	4	1	3		1	.153	.076	4.1	4.176
31																		

* Permanent teeth are numbered consecutively from upper right third molar to upper left third molar, then from lower left to lower right third molar. A similar procedure is followed in numbering deciduous teeth.

** Calculations of dentinal caries includes a 10% addition in order to correct for root fragments extracted following total crown destruction.

permanent, 4.333; in the girls' deciduous, 4.026. In comparing group means, a significant, although slight, difference was found only between the boys' permanent and deciduous teeth (Comparison 1). There was no significant difference between girls' permanent and deciduous, boys' and girls' permanent, or boys'

and girls' deciduous groups (Comparisons 2, 3, and 4). The mean values for all first permanent molars and for the remainder of permanent teeth included in the study were compared in order to determine the possible influence this tooth had on the figures presented. There was no significant difference between these classified groups (Comparison 5).

TABLE III

Evaluated comparisons of tooth groups

COMPARISON	GROUPS COMPARED	TOTAL NUMBER OF ENTRIES*	MEAN	DIFFERENCE IN MEAN	STANDARD ERROR OF DIFFERENCE	SIGNIFICANCE OF DIFFERENCE
1	Boys' Permanent	69	4.378			
	Boys' Deciduous	187	4.096	.282	.131	Yes
2	Girl's Permanent	45	4.333			
	Girl's Deciduous	113	4.026	.307	.164	None
3	Boys' Permanent	69	4.378			
	Girls' Permanent	45	4.333	.045	.176	None
4	Boys' Deciduous	187	4.096			
	Girls' Deciduous	113	4.026	.070	.114	None
5	First Perm. Molar	97	4.340			
	All Other Perm.	17	4.470	.130	.194	None

Significance of difference of the means of the groups compared, obtained by the following method:

$$\sigma_{diff.} = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$$

σ_1 = standard deviation of first group
σ_2 = standard deviation of second group
N_1 = number of items in first group
N_2 = number of items in second group

A difference is considered to be statistically significant if it is twice the standard error of difference.

* Total number of entries includes teeth actually examined plus 10% to correct for root fragments extracted following total crown destruction.

DISCUSSION

From the data presented in this study it is obvious that if, in caries research dealing with tooth surfaces, as it pertains to DMF teeth, an arbitrary value is assigned to a missing tooth, a discrepancy in the value assigned can result in an erroneous over-all picture of the caries situation. In large cross-sectional studies of caries incidence, however, a correct figure for missing teeth is not as essential as in individual longitudinal studies (5). If one desires to know the actual caries experience of the individual child, it is necessary that the true carious involvement of extracted teeth be known in relation to the remaining carious and filled teeth. This has been the main objective of the present report.

There are errors other than a misstatement of the number of carious surfaces which can be expected by use of the DMF tooth surface evaluation. As regards missing teeth, some of the reasons other than extraction due to caries are (1) congenital absence, (2) extraction because of periodontal involvement, (3) unnecessary extraction due to error of operator's judgement. For this reason, it is important to make a distinction between tooth loss due to various causes under various conditions, inasmuch as the correct carious experience would not be determined if missing teeth, other than those extracted due to caries, were included in the caries calculations.

In measurement of total caries incidence in the deciduous teeth, the symbols DMF and DMF tooth surfaces have not been used extensively by investigators, because it is often difficult to determine if the teeth have been lost because of normal exfoliation, or for other reasons. Instead, the symbols DF and DF tooth surfaces have been used (5) in reference to the deciduous dentition. For an accurate picture of the total caries incidence of an individual, however, missing deciduous teeth should likewise be given proper evaluation in relation to the remaining deciduous teeth in the mouth. If and when caries indices are more generally used to include the deciduous dentition, a careful consideration of the physiological dental age of each child will be necessary. A figure for deciduous teeth has been included in this paper in the hope that future studies on dental caries will be extended to include more data from the younger age groups.

Our findings substantiate the findings of Cheyne and Drain (6), who noted that the loss of other permanent teeth is insignificant in comparison to that of the first molar. The preponderance of extracted first permanent molars included in this study is obvious, 88 out of 104 permanent teeth included being first molars (85%). It has been postulated that the carious involvement of this tooth can serve as an index to that of the entire dental structure, if one considers large groups of individuals. The mean number of carious surfaces involved in all first permanent molars is 4.340; that of all other permanent teeth studied is 4.470. There is a difference in the means of .130 surfaces. These figures show that the mean value for the number of surfaces involved with caries in extracted first molars is not significantly different from the mean value for the number of surfaces involved in all extracted permanent teeth studied. These findings add credulence to the above theory.

It must be considered that every possible effort is made to prevent tooth loss in our clinics. The problem of expediency does not enter in, as it well might in private practice. Thus, teeth are not extracted unless they are deemed impossible to save by operative procedures. The teeth, therefore, remain in the mouth for a longer period, and of course, are susceptible for a longer period to the carious process. In this respect, data from various areas undoubtedly would show some variation.

SUMMARY AND CONCLUSIONS

An attempt has been made in this study to establish by extraoral inspection, the number of surfaces involved in teeth extracted because of caries. For an

accurate caries analysis, missing teeth must have the proper evaluation in relation to the remaining carious and filled teeth. A value is presented for deciduous and permanent teeth, giving additional consideration to individual tooth types according to sex and age, hoping that standards will be set for use in future caries investigations. Because caries studies are chiefly carried out with juvenile subjects, this study was limited to patients below the age of 15 years.

The study of the various groups of extracted teeth considered in this paper, reveals a relative constancy in the amount of destruction occurring in a tooth before it is extracted.

The mean number of tooth surfaces involved with noncarious enamel lesions, enamel caries and dentinal caries, is presented for each tooth type. The mean number of surfaces involved in the boys' permanent group is 4.378; in the boys' deciduous group, 4.096; in the girls' permanent group, 4.333; in the girls' deciduous group, 4.026. In comparing group means, a significant difference is found only between the boys' permanent and deciduous groups. This difference, though significant according to the standards set up, is slight. There is no significant difference between the mean values for all first permanent molars and for the remainder of the permanent teeth included.

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THE RÔLE OF OXALATES IN RAT DENTAL CARIES[1]

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INTRODUCTION

In 1943, McClure (1) first experimentally demonstrated an *in vivo* destructive action of rat molar enamel by acid beverages and certain dilute acids. Investigations conducted by Gortner, Restarski, Bieri and McCay supported McClure's observations in the rat and hamster (2). Such enamel etching also occurred to some extent in the teeth of monkeys and dogs (3).

It was while testing the effect of oxalic acid beverages of pH 2.6 *in vivo* that Gortner, Restarski, Bieri and McCay, noticed no enamel destruction of rats' molar teeth. Instead of enamel etching, a thin film of some substance was deposited upon the crowns of the molars. Further investigations by these same workers revealed that when oxalic acid was added to other acid beverages known to cause dissolution of rats' enamel *in vivo*, no etching of enamel occurred. Similar results were obtained with dehydrated rhubarb and spinach which are known to contain oxalates in combined and uncombined form (4). Oxalic acid seemed to protect the teeth against such destructive effect by virtue of the amount of deposit upon the teeth. When the deposit was scraped off, the underlying enamel appeared normal on gross examination. Moreover, *in vitro* studies of Buonocore and Bibby, on the effect of various ions on enamel solubility, demonstrated little if any enamel dissolution by oxalic acid (5).

It has been a general clinical observation of naval dental officers attached to various stations in the South Pacific, that little dental caries but large amounts of dental calculus is characteristic in natives subsisting on native foods. This is particularly true of natives in American Samoa and the Territory of Hawaii. Among the basic native foods of these people is poi (a native preparation of taro root). Jones, Larsen and Pritchard have shown that in the older natives who eat foods containing a substantial amount of poi, little if any dental caries occurred (6). It is interesting to note that taro flour (known to contain oxalates) also produced characteristic deposits on the molars of white rats (7).

Since several investigators have observed that dental caries is seldom found under calculus in human teeth (8), and since oxalates seem to play a role in calculus deposition, an investigation was undertaken to determine the effect of these substances on the carious process.

[1] The material in this article should be construed only as the personal opinions of the writers and not as necessarily reflecting the opinion of the Navy Department. Received for publication, August 5, 1947.

PROCEDURE

One hundred and sixty-five weanling white rat litter mates were divided into 5 major groups, hereinafter identified as groups 1 to 5, inclusive. Each of these groups was further divided into 3 subgroups, hereinafter designated subgroups A, B, and C. Litter mates of random parents were used for groups 1, 2, and 3. Groups 4 and 5 were litter mate offspring from 4 pairs of brother and sister matings from the same litter (*fig. 1*).

Subgroup A in each of the major groups acted as controls and received the coarse corn caries-producing diet recommended by McClure (9). Subgroup B in the major groups received the caries diet containing a small quantity of added

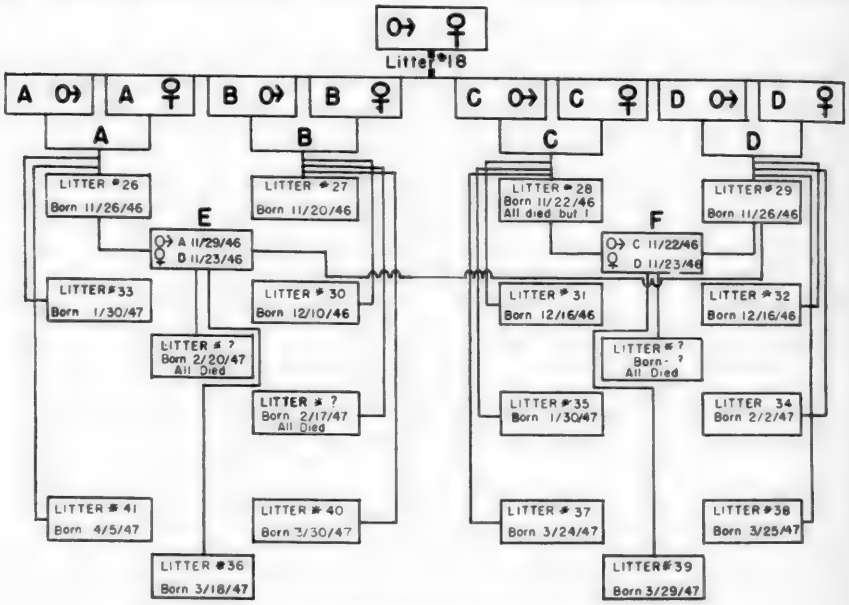

Fig. 1. White rat lineage for experimental groups 4 and 5.

oxalates in different forms. Subgroup C in the major groups received the same caries diet but with the quantity of added oxalate five to ten times that of subgroup B. Since the oxalic acid equivalent necessary to produce protection against acid beverage has been shown to be 0.11% or more (4), this quantity was accepted as a reference value from which the effect of increases or decreases in the dietary oxalates could be determined.

The oxalates were administered as follows: In group 1, subgroups B and C, the caries-producing diet contained 0.02% and 0.11% sodium oxalate. Group 2, subgroups B and C, were fed a caries-producing diet containing 0.06% and 0.33% sodium oxalate. Rats in group 3, subgroups B and C, received the oxalates in their caries diet in the form of dehydrated spinach, 1.6% and 8%, respectively, (dehydrated spinach contains about 5.88% oxalates in combined and

TABLE IA

Technique used for analysis of variance for dental caries incidence and extent (15) (16)

LITTER	DIET			TOTAL
	A	B	C	
1				ΣX_1
2				ΣX_2
3				ΣX_3
,				=
, , ,				, , ,
n				ΣX_n
Total.....	ΣX_A	ΣX_B	ΣX_C	(ΣX)

(A) Total sum of mean squares:

$$\Sigma X^2 - \frac{(\Sigma X)^2}{N}$$

(B) Sum of mean squares for diets:

$$\frac{\Sigma X_A^2 + \Sigma X_B^2 + \Sigma X_C^2}{N/3} - \frac{(\Sigma X)^2}{N}$$

(C) Sum of mean squares for litters:

$$\frac{\Sigma X_1^2 + \Sigma X_2^2 + \Sigma X_3^2 + \cdots \Sigma X_n^2}{N/n} - \frac{(\Sigma X)^2}{N}$$

Analysis of Variance

SOURCE	SUM OF MEAN SQUARES (A)	DEGREE OF FREEDOM (B)	MEAN VARIANCE (a)/(b) (C)	RATIO OF DIETS AND LITTERS TO ERROR	$F = .01$
(1) Diet.....	(B)	$b - 1$	$(B)/b - 1$	(1) (c)/(3) (c)	
(2) Litters....	(C)	$n - 1$	$(C)/n - 1$	(2) (c)/(3) (c)	
(3) Error (L × D).	$(A) - [(B) + (C)]$	$2(n - 1)$	$\frac{(A) - [(B) + (C)]}{2(n - 1)}$		
Total.....	(A)	$N - 1$			

ΣX = Sum of mean incidence or extent of caries for each litter on each diet (each cell considered separately).
(ΣX) = Total sum of mean incidence or extent of caries.
$\Sigma X_1 \ldots . n$ = Sum of mean incidence or extent of caries for each litter on all diets.
ΣX_A, ΣX_B, ΣX_C = Sum of mean incidence or extent of caries for all litters on each diet.
N = Number of cells in the block (No. of litters × No. of diets)
n = Number of litters.
b = Number of diets.
F = Value obtained from Snedecor's table of values at 1% level of probability.

uncombined form). Group 4, subgroups P and C, received Purina lab chow and 0.2% and 0.22% potassium oxalate in distilled water to drink ad libitum (which the rats did adequately), 10 days prior to being placed on a caries-producing diet, the oxalate containing water being continued for the entire experimental period. In group 5, subgroups B and C, the same regimen was followed as in group 4 except that the concentration of potassium oxalate in the drinking water was 0.0002 and 0.002%, respectively, which was consumed on the average of 50 cc. daily. The white rats remained on the caries-producing diet for 100 days, whereupon the animals were sacrificed. The heads were removed and autoclaved, after which all tissue, except the jaws with their teeth, was discarded. The jaws were placed in ether and then 95% ethanol (about 5 minutes in each). This was repeated and the jaws were dried by evaporation (10).

The teeth of each half jaw were ground in an occluso-cervical direction. A

TABLE IB

Method employed to determine significance of difference of dental caries incidence and extent in rat groups on the same caries diet (control group) (17)

$$\gamma\bar{X}_1 - \bar{X}_2 = \sqrt{\frac{(N_1 + N_2)(\Sigma X_1^2 + \Sigma X_2^2)}{N_1 N_2[(N_1 - 1) + (N_2 - 1)]}}$$ standard error of the difference of means

$$(N_1 - 1) + (N_2 - 1) =$$ Degrees of freedom

$$T = \frac{X_1 - X_2}{\gamma\bar{X}_1 - \bar{X}_2}$$ = Calculated on a 1% level

$$\Sigma X_1^2 = N_1\gamma_1^2$$

$$\Sigma X_2^2 = N_2\gamma_2^2$$

N_1—Number in 1st sample.
N_2—Number in 2nd sample.
γ—Standard deviation.

procedure of alternate grinding and inspection (recording pertinent data) was followed (10) until approximately 15 to 20 planes had been examined. In this way the caries incidence and extent could be determined through the various planes. The scoring system used to record extent of the carious lesion is a modification (10) of that adopted by Cox, Dodds, Dixon and Matuschak (11) for occlusal caries in the white rat.

The techniques (12, 13) used for analysis of variance for caries incidence and extent in the experimental rats is shown in Table IA. To determine similar differences in control groups the method shown in Table IB was employed (14).

RESULTS

In groups 1 and 2, the incidence of dental caries in 57 white rats, 100 days on a coarse corn caries-producing diet to which varying amounts of sodium oxalate had been added, ranged between a mean of 14.1 and 19.5 carious lesions. The mean total extent of these lesions varied between a score of 33.5 and 46.9 (Tables

TABLE II

Dental caries incidence and extent in white rats on varying oxalate containing caries producing diets

Age 3 weeks, duration of diet 100 days

Group 1

	A						B						C					
	CARIES DIET (CONTROL)						CARIES DIET + .02% SODIUM OXALATE						CARIES DIET + .11% SODIUM OXALATE					
	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent
	3	10	F	15	19	1.3	13	10	F	17	29	1.7	23	10	F	19	37	1.9
	4	10	M	20	35	1.7	14	10	M	17	26	1.5	24	10	F	22	33	1.5
	5	11	M	26	48	1.8	15	11	M	19	34	1.7	25	11	M	22	34	1.5
	6	11	F	24	42	1.8	16	11	F	22	37	1.6	26	11	F	22	41	1.8
	7	11	F	22	34	1.5	17	11	F	17	30	1.7	27	11	F	23	37	1.6
	91	21	M	22	43	1.9	96	21	M	0	0	0	101	21	M	18	33	1.8
	92	19	F	21	36	1.7	97	19	F	8	19	2.3	102	19	F	12	24	2.0
	93	24	M	20	45	2.2	98	24	M	14	30	2.1	103	24	M	9	31	3.4
	94	22	F	15	36	2.4	99	22	F	17	32	1.8	104	22	F	18	44	2.5
Total..	9	6		185	338	1.8	9	6		131	237	1.8	9	6		165	314	1.9
Mean..				20.6	37.6	1.8				14.6	26.3	1.8				18.3	34.9	1.9
Standard deviation.....				3.678	8.5311													
Error of mean..				1.30	3.016													

Analysis of variance for caries incidence and extent

CARIES INCIDENCE						CARIES EXTENT					
Source of variation	Sum of mean squares	Degree of freedom	Mean variance	Ratio of error to diet and litter	$F = .01$	Source of variation	Sum of mean squares	Degree of freedom	Mean variance	Ratio of error to diet and litter	$F = .01$
Diet.....	162.3	2	81.15	2.86	7.56	Diet.....	640	2	320	3.98	7.56
Litter.....	185.5	5	31.10	1.10	5.64	Litter.....	445	5	89	1.11	5.64
Error (L × D)...	283.7	10	28.37			Error (L × D)....	804	10	80.4		
Total...	631.5	17				Total....	1,889	17			

Ratio for diet = 2.86. $P > .01$ Ratio for diet = 3.98. $P > .01$
Ratio for litter = 1.10. $P > .01$ Ratio for litter = 1.11. $P > .01$
No significant difference between caries incidence or extent in rats on different diets.

II and III). The effect of adding the oxalates in the form of dehydrated spinach to the caries diet of 30 white rats in group 3 may be seen in Table IV; mean caries

TABLE III

Dental caries incidence and extent in white rats on varying oxalate containing caries producing diets

Age 3 weeks. Duration of diet 100 days

Group 2

	A						B						C					
	CARIES DIET (CONTROL)						CARIES DIET + .06% SODIUM OXALATE						CARIES DIET + .33% SODIUM OXALATE					
	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent
	31	13	F	16	41	2.5	37	13	F	19	48	2.5	43	13	F	22	64	2.9
	32	13	M	3	6	2.0	38	13	M	21	51	2.4	44	13	F	11	28	2.5
	33	14	F	24	63	2.6	39	14	F	20	53	2.7	45	14	F	25	69	2.8
	34	14	M	13	35	2.6	40	14	M	27	69	2.6	46	14	M	20	53	2.7
	35	15	F	6	12	2.0	41	15	F	21	50	2.4	47	15	F	27	70	2.6
	36	15	M	12	31	2.5	42	15	F	19	49	2.6	48	15	F	17	49	2.9
	49	16	M	21	49	1.9	53	16	M	14	33	2.4	57	16	M	13	31	2.4
	50	16	F	21	59	2.8	54	16	F	14	38	2.7	58	16	F	15	42	2.8
	51	17	M	13	32	2.4	55	17	M	17	35	2.1	59	17	M	23	47	2.0
	52	17	F	12	28	2.3	56	17	F	17	43	2.5	60	17	F	8	12	1.5
Total.	10	5		141	356	2.5	10	5		189	469	2.5	10	5		181	465	2.6
Means.				14.1	35.6	2.5				18.9	46.9	2.5				18.1	46.5	2.6
Standard deviation.....				6.641	18.350													
Error of mean..				2.213	6.117													

Analysis of variance for caries incidence and extent

CARIES INCIDENCE						CARIES EXTENT					
Source of variation	Sum of mean squares	Degree of freedom	Mean variance	Ratio of error to diet and litter	$F = .01$	Source of variation	Sum of mean squares	Degree of freedom	Mean variance	Ratio of error to diet and litter	$F = .01$
Diet.....	62.5	2	31.25	1.74	8.65	Diet.....	411	2	205	1.52	8.65
Litter.....	88.9	4	22.22	1.24	7.01	Litter....	917	4	229	1.69	7.01
Error (L × D)...	143.5	8	17.94			Error (L × D)....	1,080	8	135		
Total...	294.9	14				Total....	2,408	14			
Ratio for diet = 1.74. $P > .01$						Ratio for diet = 1.52. $P > .01$					
Ratio for litter = 1.24. $P > .01$						Ratio for litter = 1.69. $P > .01$					

No significant difference between caries incidence or extent in rats on different diets.

incidence between 17.3 and 19.5 lesions of mean total extent between a score of 33.5 and 38.1. Thirty rats in groups 4 and 5 on a caries diet who received the oxalates in their drinking water in varying amounts, showed a mean number of

TABLE IV

Dental caries incidence and extent in white rats on varying oxalate containing caries producing diets

Age 3 weeks. Duration of diet 100 days

Group 3

	A						B						C					
	CARIES DIET (CONTROL)						CARIES DIET + 1.6% DEHYDRATED SPINACH						CARIES DIET + 8% DEHYDRATED SPINACH					
	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent
	61	23	M	20	41	2.1	71	23	M	6	9	1.5	81	23	M	9	13	1.4
	62	23	M	7	14	2.0	72	23	M	15	27	1.8	82	23	M	9	13	1.4
	63	24	F	20	51	2.5	73	24	F	21	53	2.5	83	24	F	28	65	2.3
	64	24	F	23	55	2.3	74	24	F	20	51	2.5	84	24	F	22	48	2.1
	65	25	M	23	48	2.1	75	25	M	13	22	1.6	85	25	M	27	36	1.3
	66	25	M	20	41	2.1	76	25	M	25	39	1.5	86	25	M	20	30	1.5
	67	25	F	15	33	2.3	77	25	F	12	16	1.3	87	25	F	23	47	2.0
	68	25	F	17	34	2.0	78	25	F	24	40	1.6	88	25	F	18	31	1.7
	69	20	M	15	24	1.6	79	20	M	25	52	2.1	89	20	F	16	23	1.4
	70	20	M	17	40	2.3	80	20	F	12	26	2.1	90	20	F	23	37	1.6
Total..	10	4		177	381	2.2	10	4		173	335	1.9	10	4		195	343	1.6
Mean				17.7	38.1	2.2				17.3	33.5	1.9				19.5	34.3	1.6
Standard deviation.....				4.759	12.410													
Error of mean.....				1.586	4.137													

Analysis of variance for caries incidence and extent

CARIES INCIDENCE						CARIES EXTENT					
Source of variation	Sum of mean squares	Degree of freedom	Mean variance	Ratio of error to diet and litter	$F = .01$	Source of variation	Sum of mean squares	Degree of freedom	Mean variance	Ratio of error to diet and litter	$F = .01$
Diet.....	8.17	2	4.09	0.8	10.92	Diet.....	32.7	2	16.3	0.5	10.92
Litter.....	206.92	3	68.97	13.87	9.78	Litter....	1788.7	3	596.2	17.59	9.78
Error (L × D)...	29.81	6	4.97			Error (L × D)....	203.3	6	33.9		
Total...	244.92	11				Total....	2024.7	11			
Ratio for diet = 0.8. $P > .01$						Ratio for diet = 0.5. $P > .01$					
Ratio for litter = 13.87. $P < .01$						Ratio for litter = 17.59. $P < .01$					

A significant difference in caries incidence and extent in rats from different litters. No dietary influence.

carious lesions ranging between 17.9 and 21.6. Their mean total extent varied between a score of 34.2 and 43.3 (Tables V and VI). The figures given include the caries occurrence in the controls.

TABLE V

Dental caries incidence and extent in white rats on caries producing diets receiving drinking water containing varying amounts of potassium oxalate

Age 8 weeks. Duration of diet 100 days. First and second litter of control colony

Group 4

	A						B						C					
	CARIES DIET + DISTILLED DRINKING WATER (CONTROL)						CARIES DIET + .22% POTASSIUM OXALATE IN DRINKING WATER						CARIES DIET + .02% POTASSIUM OXALATE IN DRINKING WATER					
	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent
	106	26	M	13	25	1.9	118	26	M	17	37	2.1	112	26	M	22	49	2.2
	107	26	F	20	37	1.8	119	26	F	19	40	2.1	113	26	F	25	49	2.1
	111	26	F	14	25	1.7	123	26	F	30	64	2.1	117	26	F	26	58	2.2
	108	27	M	27	49	1.8	120	27	F	29	62	2.1	114	27	M	3	6	2.0
	109	27	F	27	65	2.4	121	27	F	25	54	2.1	115	27	F	21	41	1.9
	110	29	M	30	68	2.2	122	29	M	27	59	2.1	116	29	M	29	67	2.3
	124	30	M	21	41	1.9	132	30	M	21	40	1.9	128	30	M	21	44	2.1
	125	30	F	25	48	1.9	133	30	F	22	50	2.2	129	30	F	24	56	2.3
	148	30	M	20	38	1.9	152	30	M	20	34	1.7	150	30	M	17	33	1.9
	126	31	M	11	21	1.9	134	31	M	19	37	1.9	130	31	M	11	15	1.3
	127	31	F	22	52	2.3	135	31	F	14	26	1.8	131	31	F	9	17	1.8
	149	31	F	21	48	2.2	153	31	F	22	50	2.2	151	31	F	12	21	1.7
	136	32	M	16	20	1.2	140	32	M	17	26	1.5	138	32	M	25	40	1.6
	137	32	F	12	21	1.7	141	32	F	29	54	1.8	139	32	M	15	32	2.1
	142	32	M	13	19	1.4	146	32	M	16	26	1.6	144	32	M	22	38	1.7
	143	32	F	17	25	1.4	147	32	M	19	34	1.7	145	32	M	22	49	2.2
Total	16	6		309	602	1.95	16	6		346	693	2.0	16	6		304	615	2.0
Mean				19.3	37.6	1.95				21.6	43.3	2.0				19.0	38.4	2.0
Standard deviation				5.918	16.161													
Error of mean				1.530	4.177													

Analysis of variance for caries incidence and extent

CARIES INCIDENCE						CARIES EXTENT					
Source of variation	Sum of mean squares	Degree of freedom	Mean variance	Ratio of error to diet and litter	$P = .01$	Source of variaton	Sum of mean squares	Degree of freedom	Mean variance	Ratio of error to diet and litter	$P = .01$
Diet (water oxalate)	27	2	13.5	.62	7.56	Diet (water oxalate)	93	2	47	.31	7.56
Litter	280	5	56.0	2.57	5.64	Litter	2,167	5	433	2.91	5.64
Error (L × D)	218	10	21.8			Error (L × D)	1,492	10	149		
Total	525	17				Total	3,752	17			

Ratio for diet (oxalate in water) = .62. $P > .01$

Ratio for litter (oxalate in water) = 2.57. $P > .01$

Ratio for diet (oxalate in water) = .31. $P > .01$

Ratio for litter (oxalate in water) = 2.91. $P > .01$

No significant difference between caries incidence or extent in rats on caries diets receiving oxalate in their drinking water.

TABLE VI

Dental caries incidence and extent in white rats on caries producing diets receiving drinking water containing varying amounts of potassium oxalate

Age: 3 weeks; duration of diet 100 days. Second and third litters of control colony

Group 5

	A — CARIES DIET + DISTILLED DRINKING WATER (CONTROL)						B — CARIES DIET + .0002% POTASSIUM OXALATE IN DRINKING WATER						C — CARIES DIET + .002% POTASSIUM OXALATE IN DRINKING WATER					
	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent	Rat no.	Litter no.	Sex	Caries incidence	Total extent	Average extent
	154	33	M	25	54	2.1	156	33	M	13	16	1.2	158	33	M	4	8	2.0
	155	33	M	18	34	1.8	157	33	F	15	26	1.7	159	33	F	19	44	2.3
	160	33	M	14	18	1.2	162	33	M	15	28	1.8	164	33	M	22	49	2.2
	161	33	F	24	40	1.6	163	33	F	23	48	2.08	165	33	F	22	45	2.04
	166	34	M	28	63	2.2	168	34	M	22	48	2.1	170	34	M	9	11	1.2
	167	34	F	19	43	2.2	169	34	F	18	41	2.2	171	34	F	20	30	1.5
	179	34	F	19	41	2.1	181	34	F	17	24	1.4	183	34	F	22	40	1.8
	172	35	M	13	23	1.7	174	35	M	20	42	2.1	176	35	M	19	33	1.7
	173	35	F	25	58	2.3	175	35	F	19	36	1.8	177	35	F	25	47	1.8
	178	35	F	21	46	2.1	180	35	F	17	33	1.9	182	35	F	27	59	2.1
Total..	10	3		206	420	2.04	10	3		179	342	1.91	10	3		189	366	1.94
Mean..				20.6	42.0	2.04				17.9	34.2	1.91				18.9	36.6	1.94
Standard deviation.....				4.673	13.638													
Error of mean...				1.558	4.546													

Analysis of variance for caries incidence and extent

CARIES INCIDENCE						CARIES EXTENT					
Source of variance	Sum of mean squares	Degrees of freedom	Mean variance	Ratio of error to diet and litter	$P = .01$	Source of variance	Sum of mean squares	Degrees of freedom	Mean variance	Ratio of error to diet and litter	$P = .01$
Diet (water oxalate)....	3	2	1.5	.19	18.00	Diet (water oxalate)...	39	2	19.5	.58	18.00
Litter.....	6	2	3.0	.39	18.00	Litter....	90	2	45.0	1.33	18.00
Error (L × D)...	31	4	7.75			Error (L × D)...	135	4	33.75		
Total...	40	8				Total....	264	8			

Ratio for diet (oxalate in water) = .19. $P > .01$

Ratio for litter (oxalate in water) = .39. $P > .01$

Ratio for diet (oxalate in water) = .58. $P > .01$

Ratio for litter (oxalate in water) = 1.33. $P > .01$

No significant difference between dental caries incidence and extent in rats on caries diet receiving the oxalates in drinking water.

After applying a technique for analysis of variance (Table IA) to the results obtained in the different groups of experimental animals, it became evident that the oxalates as given exerted no influence on the dental caries experience. In group 3 (dehydrated spinach, Table IV) the significant difference in the caries incidence and extent between rats on the different diets was accountable on a hereditary rather than dietary basis upon statistical analysis. In none of the

TABLE VII

Differences in dental caries incidence and extent in white rat controls of study groups 1, 2, 3, 4, 5, on the same caries producing diet 100 days (formula Table IB)

DENTAL CARIES INCIDENCE				DENTAL CARIES EXTENT		GROUPS ANALYZED	ERROR	DIFFERENCE/ERROR	DEGREES OF FREEDOM	$T = 1\%$ LEVEL	$T = 5\%$ LEVEL
Study groups	Number of rats	Mean	Standard deviation	Mean	Standard deviation						
						Incidence					
1	9	20.6	3.678	37.6	8.531	1 and 2	2.642	2.460	17	2.898	2.110
2	10	14.1	6.641	35.6	18.350	1 and 3	2.079	.140	17	2.898	2.110
3	10	17.7	4.759	38.1	12.410	1 and 4	2.267	.573	21	2.807	2.069
4	16	19.3	5.918	37.6	16.161	1 and 5	2.054	0.000	17	2.898	2.110
5	10	20.6	4.673	42.0	13.638	2 and 3	2.722	1.323	18	2.878	2.101
						2 and 4	2.604	1.998	24	2.797	2.064
						2 and 5	2.707	2.401	18	2.878	2.101
						3 and 4	2.307	.697	24	2.797	2.064
						3 and 5	2.222	1.305	18	2.878	2.101
						4 and 5	2.295	.566	24	2.797	2.064
						Extent					
						1 and 2	7.069	.283	17	2.898	2.110
						1 and 3	5.130	.098	17	2.898	2.110
						1 and 4	6.044	.000	23	2.807	2.069
						1 and 5	5.593	.787	17	2.898	2.110
						2 and 3	2.337	1.070	18	2.878	2.101
						2 and 4	7.153	.280	24	2.797	2.064
						2 and 5	7.625	.839	18	2.878	2.101
						3 and 4	6.230	.0803	24	2.797	2.064
						3 and 5	6.157	.633	18	2.878	2.101
						4 and 5	6.399	.688	24	2.797	2.064

A significant difference in caries incidence is noted between groups (1 and 2), (2 and 5) at the 5% level but not at the 1% level of probability.

other major groups of experimental animals could an hereditary influence on the occurrence of dental caries be demonstrated statistically. Neither the concentration of the oxalates in the diet (0.02% to 0.33%) nor in the drinking water (1:1,000 to 1:1,000,000 oxalic acid equivalent) seemed to have any effect on caries in the groups of white rats studied.

No significant difference could be found between the caries occurrence in those 5 subgroups A of white rats which were used as controls, all of which subgroups were on the same caries diet (*fig. 1B* and Table VII). The mean carious lesions

ranged between 14.1 and 20.6 and their total extent varied between a mean score of 35.1 and 42.0.

Many carious lesions were found immediately under the dental calculus-like deposits within the coronal grooves. That these lesions may have been extensions from other planes reaching areas immediately under the deposits could be dismissed since the system of alternate grinding and inspection through all planes precluded such a possibility.

DISCUSSION

In this study it is evident that the oxalates in the dietary are not protective against the dental caries process as induced by a caries diet in white rats. Similar incomplete unpublished data, thus far accumulated on cotton rats here studied, tend to show the same results, although it is not implied that similar results are to be expected in humans.

The clinical differences between acid etching or erosion, and dental caries have been generally observed by practicing dentists and investigators working with experimental animals. Grossly reviewed, these are the loss of tooth substance leaving a hard polished exposed surface discolored to varying degrees and depth in erosion or acid etching, as compared to the chalky appearance and hardness of initial carious lesions progressing to the leathery, discolored, extensive destruction of tooth substance in advanced dental caries. Since the oxalates have been shown to be protective against etching of rat molars by acid beverages (4), and not against the carious process, it appears that this study is supportive evidence in the differentation between acid erosion and dental caries.

The fact that the "oxalate" deposits in the coronal grooves of the experimental rats did not influence the carious process, and that caries was seldom found under human dental calculus (8), raises a question of chemical composition of both types of deposits. Such comparisons have as yet not been made. It is possible that the chemical composition of the "oxalate" deposits may not be the same as other dental calculi; and that, consequently, some factor or factors inhibiting dental caries under human dental calculus may be absent under the "oxalate" deposits. Unless, of course, we may be ignoring a physico-chemical mechanism which might be operating peculiar to rat molars and associated with their external configuration.

Concerning the mechanism involved in the process of initial dental caries, there appear to be two schools of thought. The one headed by Miller (15) who contended that decalcification of enamel caused by products of oral acidogenic organisms, occurs first and is followed by proteolysis of the inorganic content of enamel or dentin by oral proteolytic bacteria. The other school headed by Hopewell-Smith (16) considered the sequence of events to be proteolysis of the enamel cuticle (Nasmyth's membrane) by oral proteolytic organisms followed by the decalcification process in the enamel by acidogenic bacteria. The results of the study on rats here undertaken would tend to minimize the acidogenic factor as most important in the initial stages of the dental caries process. Were the acid dissolution of the enamel of greatest importance in the initial stages of

dental caries in general, then the protective action of the oxalates should have reduced caries occurrence and extent in a manner similar to the way they protected the enamel against the solvent effect of acid beverages shown in other studies (4). As has been shown, the dietary oxalates did not influence dental caries in rat molars.

From this work it becomes obvious that further work will be necessary to more fully evaluate the factors tending to operate in initial dental caries.

SUMMARY AND CONCLUSIONS

1. The oxalates administered in the diet and drinking water in this study produced no significant effect on the dental caries incidence and extent in 5 groups of white rat litter mates fed a coarse corn caries-producing diet.

2. Although considerable dental calculus-like deposits occurred within the grooves of the experimental rats' teeth, they did not appear to influence dental caries occurrence in these susceptable areas.

3. This work tends to minimize the importance of the acidogenic factor in the initial stages of the dental caries process.

4. Further in vivo studies in this problem seem indicated to determine if any variations exist in different species and different time intervals of application of oxalates prior to placing animals on a caries producing diet.

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